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(54) Title: IDURONIDASE-CLEAVABLE COMPOUNDS

(57) Abstract: The present disclosure relates to novel, iduronidase-cleavable compounds and related methods. Moreover, the present disclosure relates to pharmaceutical compositions comprising such a compound and the use of such compounds in medical treatment methods. Furthermore, the present disclosure relates to methods of increasing the cytotoxicity of a molecule and methods of reducing cellular proliferation.

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IDURONIDASE-CLEAVABLE COMPOUNDS

FIELD OF THE INVENTION

The present disclosure relates to novel, iduronidase-cleavable compounds and related methods. Moreover, the present disclosure relates to pharmaceutical compositions comprising such a compound and the use of such compounds in medical treatment methods. Furthermore, the present disclosure relates to methods of increasing the cytotoxicity of a molecule and methods of reducing cellular proliferation.

BACKGROUND OF THE INVENTION

Antibody-drug conjugates (ADC) combine the specificity of a monoclonal antibody with the cytotoxicity of a potent small-molecule warhead (Beck et al., 2017; de Goeij and Lambert, 2016). In that manner, ADCs represent the next-generation of targeted chemotherapy with currently three FDA-approved molecules available on the market.

Their high anti-tumor efficacy of ADCs, however, often comes at the cost of dose-limiting toxicities (DLT) that are frequently observed during clinical development (Donaghy, 2016). Those DLTs include severe neutropenia and thrombocytopenia as well as ocular toxicity or peripheral neuropathy, to name a few (Masters et al., 2018; Uppal et al., 2015; Zhao et al., 2017). While some of these DLTs can be associated with the antibody portion of an ADC due to the expression of the respective antigen on healthy tissue, others can be attributed to the linker-payload combination. Common strategies to overcome DLTs can range from an adjusted dosing regimen in the clinic to drug engineering efforts that are conducted at the drug discovery stage.

In general, antibody-drug conjugates can be equipped either with a cleavable or a non-cleavable linker (Tsuchikama and An, 2018). While non-cleavable linkers exhibit improved stability in the circulation, they have been reported to be less efficient due to the lack of a bystander effect (Hamblett et al., 2015). In addition, linker sequences that can be cleaved by lysosomal enzymes

can offer the inherent advantage of a selective cleavage in tumor tissues in contrast to normal tissue if the enzyme exhibits a differential expression profile.

The most commonly employed enzyme-cleavable linker sequence is the valine-citrulline peptide sequence that is cleaved by cathepsin B (Chari et al., 2014; Dubowchik and Firestone, 1998). However, while overexpressed in a variety of different tumor types, cathepsin B is also highly expressed in a plethora of normal cells and immune cells. In combination with a tubulin-inhibiting agent such as monomethyl auristatin E (MMAE), the clinical development of cathepsin B-cleavable ADCs is often hampered by severe neutropenia (Donaghy, 2016). This can potentially be attributed to the presence of cathepsin B in neutrophil progenitor cells. Once the ADC is taken up non-specifically by a rapidly dividing progenitor cell, the linker portion can be cleaved by cathepsin B and the MMAE warhead is released. Another cause for neutropenia is the instability of the commonly used Val-Cit linker in circulation (Donaghy, 2016). Interestingly, it was observed that the Val-Cit linker system is susceptible to enzyme-mediated hydrolysis in mouse and rat plasma and that rodent-specific carboxylesterase 1c (CES1c) is responsible for linker-drug instability (M. Dorywalska et al., 2016; R. Ubink et al., 2018). These findings motivated Dorywalska et al. to generate acetylated lysin derivatives of the Val-Cit linker system (e.g., K(Ac)-VC-linker) that showed resistance to CES1c-cleavage but not to degradation via lysosomal protease. In addition, not only off-target toxicity but also on-target toxicity can occur when the enzyme mediating the linker cleavage is highly expressed in the respective tissue.

Accordingly, there is a need in the art for improved ADCs. Moreover, there is a need in the art for improved linkers for ADCs. Moreover, there is a need in the art for ADCs with improved tolerability and/or efficacy. Moreover, there is a need in the art for linkers with improved stability in the circulation and/or inside normal healthy cells. Moreover, there is a need in the art for linkers which allow for effective drug release inside diseased tissues. Moreover, there is a need in the art for ADCs with low tendency to undergo aggregation. Moreover, there is a need in the art for linkers that address these problems and that at the same time are inexpensive and allow for fast synthetic access. Moreover, there is a need in the art for linkers that, if used in an ADC context, combine any or all of the above characteristics.

The present disclosure overcomes the above-described problems and addresses the above-described needs.

SUMMARY OF THE INVENTION

The present disclosure addresses the needs described above in the section "Background of the Invention" by the different aspects and embodiments described below.

Linkers must be stable in the circulation and efficiently release the drug payload inside target cells. Thus, linker characteristics have a great impact on ADC pharmacokinetics and efficacy. The present invention is, in part, based on the surprising observation that the novel linkers of the present disclosure, in which hydrolysis by lysosomal α-L-iduronidase (IDUA) releases the active drug of the compound, allow for the generation of ADCs with superior characteristics. The compounds according to the present disclosure were found to provide high serum stability, target-dependent cell killing in the subnanomolar range and, compared to analogous compounds with a peptide-based linker, reduced toxic activity on normal cells. This implies a high stability of the novel linkers in the systemic circulation and in normal cells while still allowing effective cleavage in tumor cells, indicating reduced toxic side effects by ADCs utilizing the iduronide-based linkers of the present disclosure. *In vivo* evaluation of an iduronidase-cleavable ADC according to the present disclosure showed high antitumor activity. Thus, the studies reported herein demonstrate that the iduronide-based linkers according to the present disclosure provide a very advantageous combination of high efficacy and superior safety benefits.

In an aspect, the present disclosure relates to a compound comprising a Linker unit, wherein said Linker unit has a structure according to the formula

$$A_a\text{–}W_w\text{–}Y_y \qquad \text{(Formula 1)},$$

wherein:

A is a Stretcher unit;

a is 0, 1 or 2;

W is an Iduronide unit;

w is 1 or 2;

Y is a Spacer unit;

y is 0, 1 or 2.

In another aspect, the present disclosure relates to a compound comprising a Linker-Functional agent unit, wherein said Linker-Functional agent unit has a structure according to the formula

$$A_a–W_w–Y_y–F_f \qquad \text{(Formula 3)},$$

wherein:

A is a Stretcher unit;

a is 0, 1 or 2;

W is an Iduronide unit;

w is 1 or 2;

Y is a Spacer unit;

y is 0, 1 or 2;

$A_a–W_w–Y_y$ is a Linker unit;

F is a Functional agent;

f is 1, 2, 3 or 4.

In another aspect, the present disclosure relates to a compound having a structure according to the formula

$$\text{Ligand}–(–A_a–W_w–Y_y–F_f)_p \qquad \text{(Formula 5)},$$

wherein:

Ligand is a Ligand unit;

A is a Stretcher unit;

a is 0, 1 or 2;

W is an Iduronide unit;

w is 1 or 2;

Y is a Spacer unit;

y is 0, 1 or 2;

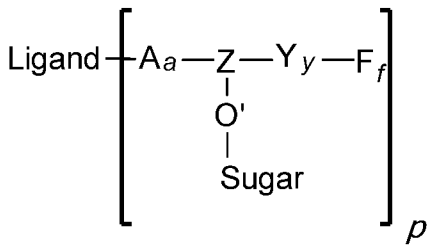
A_a–W_w–Y_y is a Linker unit;

F is a Functional agent;

f is 1, 2, 3 or 4;

p is from 1 to 20.

In another aspect, the present disclosure relates to a compound having a structure according to the formula



(Formula 8),

wherein:

Ligand is a Ligand unit;

A is a Stretcher unit;

a is 0, 1 or 2;

Z–O'–Sugar is an Iduronide unit ("W"),

wherein

Z is a self-immolative group,

–O'– is a glycosidic bond,

Sugar is a sugar moiety;

A_a–[Z-O'-Sugar]–Y_y is a Linker unit;

Y is a Spacer unit;

y is 0, 1 or 2;

F is a Functional agent;

f is 1, 2, 3 or 4;

p is from 1 to 20.

In another aspect, the present disclosure relates to a method for preparing a compound according to the present disclosure, wherein said method comprises the step of covalently linking at least one molecule comprising a Functional agent to a molecule comprising a Ligand unit.

In another aspect, the present disclosure relates to a molecule for use in the preparation of a compound according to the present disclosure, wherein said molecule comprises an Iduronide unit that is covalently linked to an activator group.

In another aspect, the present disclosure relates to the use of a molecule in the preparation of a compound according to the present disclosure, wherein said molecule comprises an Iduronide unit covalently linked to an activator group.

In another aspect, the present disclosure relates to a method for increasing the cytotoxicity of a molecule, said molecule comprising a Ligand unit, wherein said method comprises covalently linking at least one Functional agent to said molecule, thus providing a compound according to the present disclosure, wherein said Functional agent is a cytotoxic agent (i.e. a therapeutic agent which is a cytotoxic agent).

In another aspect, the present disclosure relates to a method for increasing the cytotoxicity of a molecule, said molecule comprising a Ligand unit, but no Functional agent, wherein said method comprises the preparation of a compound according to the present disclosure, wherein said Functional agent is a cytotoxic agent (i.e. a therapeutic agent which is a cytotoxic agent).

In another aspect, the present disclosure relates to the use of a molecule comprising a Functional agent for increasing the cytotoxicity of a molecule comprising a Ligand unit, wherein said use involves the step of covalently linking at least one molecule comprising a Functional agent to said molecule comprising a Ligand unit, thus providing a compound according to the present disclosure, wherein said Functional agent is a cytotoxic agent (i.e. a therapeutic agent which is a cytotoxic agent).

In another aspect, the present disclosure relates to a pharmaceutical composition comprising the compound according to the present disclosure.

In another aspect, the present disclosure relates to a method for treating a disease in a patient in need thereof, comprising the step of administering to said patient a therapeutically effective amount of the compound according to the present disclosure or the pharmaceutical composition according to the present disclosure.

In another aspect, the present disclosure relates to the use of the compound according to the present disclosure or the pharmaceutical composition according to the present disclosure for the manufacture of a medicament.

In another aspect, the present disclosure relates to the use of the compound according to the present disclosure or the pharmaceutical composition according to the present disclosure for the manufacture of a medicament for the treatment of cancer.

In another aspect, the present disclosure relates to the use of the compound according to the present disclosure or the pharmaceutical composition according to the present disclosure for the manufacture of a medicament for the treatment of a malignant tumor.

In another aspect, the present disclosure relates to a method of reducing cellular proliferation, the method comprising contacting a cell with an anti-proliferative amount of a compound according to the present disclosure or with an anti-proliferative amount of a pharmaceutical composition according to the present disclosure.

BRIEF DESCRIPTION OF THE FIGURES

In the following, reference is made to the figures. All methods referred to in the figure descriptions below were carried out as described in detail in the examples.

Figure 1 shows data from a recombinant α-L-iduronidase cleavage assay and α-L-iduronidase mRNA expression data. **A)** Recombinant α-L-iduronidase cleavage assay with fluorogenic substrates for α-L-iduronidase (4-methylumbelliferyl α-L-iduronide (4-MU)), β-D-glucuronidase (4-methylumbelliferyl-β-D-glucuronide), β-galactosidase (fluorescein di(β-D-galactopyranoside)). RFU, relative fluorescence units. **B)** α-L-iduronidase mRNA expression derived from the database *GTEx* and encompassing endothelial cells of different normal tissues,

human umbilical vein endothelial cells (HUVEC) and **C)** immune cells in comparison with cathepsin B. TPM, transcript reads per million reads mapped. Blue bars (left): Expression of cathepsin B. Black bars (right): Expression of iduronidase. Bar charts indicate sample means. **D)** α-L-iduronidase mRNA expression in primary human tumors (stomach adenocarcinoma (STAD), colon adenocarcinoma (COAD), pancreatic adenocarcinoma (PAAD), liver hepatocellular carcinoma (LIHC), cholangiocarcinoma (CHOL), from the database TCGA in comparison with matched normal tissues. TPM, transcript reads per million reads mapped. Red horizontal lines indicate the sample means.

Figure 2 depicts the synthesis route for the MC-iduronide-exatecan (**12**) Linker-Payload. A) First part of synthesis route from compound (**2**) and (**3**) to compound (**7**). **B)** Continuation of synthesis from compound (**7**) to compound (**12**). The synthesis of the protected iduronic acid precursor (**2**) can be found in the literature (Lu et al., 2013). 4-(1,3-dioxolan-2-yl)-2-nitrophenol (**3**) was glycosylated with the protected iduronic acid precursor *via* a Mitsunobu reaction. The acetal protecting group was removed under acidic conditions and the resultant aldehyde was reduced to the primary alcohol (**5**) using sodium borohydride. In the next step, the nitro group was catalytically hydrated followed by coupling of the resultant amine to Fmoc protected glycine. To attach exatecan (**8**) to the linker portion *via* a carbamate functionality, the primary alcohol (**6**) was activated and reacted with the secondary amine of exatecan (**8**). Subsequently, acetyl, methoxy and Fmoc protecting groups were removed under basic conditions and in the last step the maleimide handle was attached to yield MC-iduronide-exatecan (**12**).

Figure 3 provides the structures of linker-payload compounds used for ADC generation.

Figure 4 shows data from an experiment to characterize the release kinetics of ADCs with iduronide linker**.** (**A**) Payload release mechanism of N-acetylcysteine-quenched NAC-MC-iduronide-exatecan (**16**). (**B**) Release kinetics of NAC-MC-iduronide-exatecan (**16**) and NAC-MC-K(Ac)-VC-exatecan (**18**) using recombinant human α-L-iduronidase (IDUA) and recombinant human cathepsin B (CatB). Overall, 5 µM of linker-payload were incubated with 0.25 ng/µL enzyme at the pH optimum and 37 °C for 3h and the reaction was analyzed by LC-MS every 12 min. (**C**) pH dependency of IDUA and CatB enzymatic cleavage rates. Enzymatic cleavage rates (k_{enz}) were obtained by fitting the decrease of parent species (**16**) and (**18**) by a non-linear regression (one-phase-decay) in GraphPad Prism.

Figure 5 summarizes results from an experiment to study *in vitro* exatecan release from unconjugated linker-payload structures in cell lysates or lysosomal extract from different cell types. 5 µM quenched linker-payload was incubated together with 5 µM 7-Ethyl-d3-camptothecin (D3-CPT) internal standard in 20 µg cell lysate and catabolism buffer (pH 5.0) at 37 °C for up to 48 h. Free exatecan was quantified by LC-MS/MS and is depicted as %total exatecan (5 µM = 100%). **A)** MC-iduronide-exatecan and MC-K(Ac)VC-exatecan incubated in cell lysates from mononuclear cells (MNC), peripheral blood mononuclear cells (PBMC), normal human dendritic cells (NHDC), normal human epidermal keratinocytes (NHEK) and liver lysosomes. **B)** MC-iduronide-exatecan and MC-AAN-exatecan are also known as substrates for lysosomal legumain and cathepsin B in human umbilical vein (HUVEC) lysates. Enhanced exatecan release from MC-K(Ac)VC-exatecan or MC-AAN-exatecan compared to MC-iduronide-exatecan reflects reduced IDUA concentration in cell lysates and lysosomal extracts.

Figure 6 depicts the concept of ADC generation in Example 6 and summarizes experimental results obtained from the characterization of ADCs. (**A**) Iduronide-exatecan conjugated *via* its maleimide handle to all 8 interchain cysteine residues aimed at a DAR of 8. (**B**) Characteristics of the generated exatecan ADCs for this study. ADCs differ in their cleavable linker motif: i) iduronidase-cleavable iduronide linker, ii) cathepsin B-cleavable valine-citrulline linker and iii) legumain and cathepsin B-cleavable alanine-alanine-asparagine linker. **(C)** Serum stability of aCEA5(anti-CEACAM5)-iduronide-exatecan. Free exatecan was measured via LC MS/MS after incubation in mouse, cynomolgus and human sera at 37 °C for 96 h. No free exatecan was detected indicating that iduronide-exatecan is not cleaved by serum proteases in these species.

Figure 7 summarizes *in vitro* cell viability data. **A)** Cell viability on CEA5 positive (SK-CO-1, MKN-45, LS174T) and CEA5 negative cells (MDA-MB-231). Cells were incubated with serial dilution of ADCs and free exatecan for 6 days before cell viability was analyzed. Error bars represent standard deviation (SD) of triplicates. (**B**) Inhibitory activity of ADCs and free exatecan. IC50 values are given as mean of three independent experiments (IC_{50} ± SD).

Figure 8 shows data confirming the broad applicability of the iduronide-cleavable linker upon conjugation to additional monoclonal antibodies targeting EGFR (matuzumab). (**A**) The resulting iduronide-exatecan ADCs demonstrated excellent stability in mouse serum. (**B**) For matuzumab-based ADCs, potent cell killing was observed in *in vitro* cytotoxicity assays.

Figure 9 shows data for the *in vitro* release of exatecan from unconjugated linker-payload structures in primary normal human epidermal keratinocytes (NHEK) cell lysates as model system for ADC Payload Linker cleavage in normal epidermal cells. Overall, 5 µM of quenched MC-iduronide-exatecan and MC-K(Ac)VC-exatecan were incubated together with 5 µM 7-Ethyl-d3-camptothecin (D3-CPT) internal standard in 20 µg cell lysate and catabolism buffer (pH 5.0) at 37 °C for up to 48 h. Free exatecan was quantified by LC-MS/MS and is given as %total exatecan (5 µM = 100%). Enhanced exatecan release from MC-K(Ac)VC-exatecan or MC-AAN-exatecan compared to MC-iduronide-exatecan reflects reduced IDUA concentration in cell lysates.

Figure 10 shows data confirming that iduronide linker can be applied with different warheads to generate functional ADCs. An aCEA5 antibody was conjugated at position Q295 to the MC-iduronide-duocarmycin (**15**) linker-payload. The resulting ADC showed selective cell killing of CEA5 positive MKN45 cells over CEA5 negative MKN45 CEA5 knock-out cells, while the free duocarmycin payload shows similar IC50 values on both cell lines irrespective of CEA5 expression status.

Figure 11 shows data from experiments to analyze toxicity in different cell types. *In vitro* cell toxicity models were utilized to mimic **(A)** liver toxicity via mono- and co-cultures of primary hepatocytes and liver sinusoidal endothelial cells (LSECs). Cells were treated with 900 nM of each ADC (or 250 nM of exatecan) initially and re-treated after 3 days (medium exchange). ATP was measured after 6 days *via* a luminescence read-out. (**B**) HUVEC cells were used to assess toxicity on endothelial cells. HUVEC cells were seeded (1000 cells/well) and treated with ADCs or free exatecan at 25 nM for 6 days at 37 °C, 5% CO_2 before cell viability was analyzed via a luminescence read-out.

Figure 12 shows results from *in vivo* experiments to characterize the antitumor activity of an iduronidase-cleavable aCEA-duocarmycin ADC. A single dose of iduronide duocarmycin ADC (5 mg kg-1, square) and vehicle control (circle) were administered to SNU-16 subcutaneous tumor bearing H2d Rag2 mice when a mean tumor volume reached ~150-200 mm^3. Error bars represent s.e.m.

Figure 13 shows how the Stretcher unit, Iduronide unit, Spacer unit, self-immolative group and Functional agent can be reflected in the chemical structure of a compound according to Formula 53.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Although the present disclosure is described in detail above and below, it is to be understood that this disclosure is not limited to the particular methodologies, protocols and reagents described by the present disclosure, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present disclosure which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, certain elements of the present disclosure will be described in more detail, including the description of specific embodiments. However, the variously described examples and preferred embodiments should not be construed to limit the present disclosure to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements and in any manner. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application except for where this leads to logical contradictions or the context indicates otherwise.

Unless defined otherwise herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclatures and techniques referred to in the present disclosure, e.g. nomenclatures and techniques of organic chemistry, chemical synthesis, biology, medicinal and pharmaceutical chemistry, medicine, pharmacology or toxicology, are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in the references cited and discussed throughout the present disclosure unless otherwise indicated.

First aspect of the present disclosure (also referred to as "Embodiment 1"): According to a first aspect, the present disclosure relates to a compound comprising a Linker unit, wherein said Linker unit has a structure according to the formula

$$A_a-W_w-Y_y \qquad \text{(Formula 1)},$$

wherein:

A is a Stretcher unit;

a is 0, 1 or 2;

W is an Iduronide unit;

w is 1 or 2;

Y is a Spacer unit;

y is 0, 1 or 2.

If the Linker unit is part of a larger compound, the Linker unit is typically connected to further components of said compound (e.g. a Ligand unit and/or a Functional agent) by covalent bonds, usually by

- a covalent bond linked to the Spacer unit (if a Spacer unit is present) or to the Iduronide unit (if no Spacer unit is present) and
- a covalent bond linked to the Stretcher unit (if a Stretcher unit is present) or to the Iduronide unit (if no Stretcher unit is present).

Such covalent bonds are indicated in Formula 2 below.

Embodiment 2: The compound according to embodiment 1, wherein said Linker unit has a structure according to the formula

$$-A_a-W_w-Y_y- \qquad \text{(Formula 2)}.$$

Second aspect of the present disclosure (also referred to as "Embodiment 3"): According to a second aspect, the present disclosure relates to a compound comprising a Linker-Functional agent unit, wherein said Linker-Functional agent unit has a structure according to the formula

$$A_a\text{–}W_w\text{–}Y_y\text{–}F_f \quad \text{(Formula 3),}$$

wherein:

A is a Stretcher unit;

a is 0, 1 or 2;

W is an Iduronide unit;

w is 1 or 2;

Y is a Spacer unit;

y is 0, 1 or 2;

A_a–W_w–Y_y is a Linker unit;

F is a Functional agent;

f is 1, 2, 3 or 4.

If the Linker-Functional agent unit is part of a larger compound, then the Linker-Functional agent unit is typically connected to at least one further component of said compound (e.g. a Ligand unit) by a covalent bond, usually by a covalent bond linked to the Stretcher unit (if a Stretcher unit is present) or to the Iduronide unit (if no Stretcher unit is present) of said Linker-Functional agent unit.

Such covalent bond is indicated in Formula 4 below.

Embodiment 4: The compound according to embodiment 3, wherein said Linker-Functional agent unit has a structure according to the formula

$$-A_a\text{–}W_w\text{–}Y_y\text{–}F_f \quad \text{(Formula 4).}$$

Third aspect of the present disclosure (also referred to as "Embodiment 5"): According to a third aspect, the present disclosure relates to a compound having a structure according to the formula

$$\text{Ligand}\text{–}(\text{–}A_a\text{–}W_w\text{–}Y_y\text{–}F_f)_p \quad \text{(Formula 5),}$$

wherein:

Ligand is a Ligand unit;

A is a Stretcher unit;

a is 0, 1 or 2;

W is an Iduronide unit;

w is 1 or 2;

Y is a Spacer unit;

y is 0, 1 or 2;

A_a–W_w–Y_y is a Linker unit;

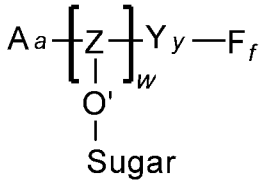
F is a Functional agent;

f is 1, 2, 3 or 4;

p is from 1 to 20.

As the skilled person understands, A_a–W_w–Y_y–F_f is a Linker-Functional agent unit.

Embodiment 6: The compound according to any one of embodiments 3 to 5, wherein said Linker-Functional agent unit has a structure according to the formula



(Formula 6),

wherein

Z–O'–Sugar is an Iduronide unit ("W"),

wherein

Z is a self-immolative group,

–O'– is a glycosidic bond,

Sugar is a sugar moiety;

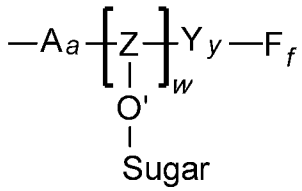
A_a–[Z-O'-Sugar]–Y_y is a Linker unit.

The group [Z-O'-Sugar] in Formula 6 and other formulas may be referred to as "W" (i.e. capital non-italic W). In the definition above, the "*w*" following the square bracket is a lowercase, italic *w*. This is to be distinguished from the uppercase, non-italic "W" that designates the complete Iduronide unit Z–O'–Sugar above and also in other formulas herein, in e.g. Formula 3.

If the Linker-Functional agent unit is part of a larger compound, then the Linker-Functional agent unit is typically connected to at least one further component of said compound (e.g. a Ligand unit) by a covalent bond, usually by a covalent bond linked to the Stretcher unit (if a Stretcher unit is present) or to the Self-immolative group Z of the Iduronide unit (if no Stretcher unit is present) of said Linker-Functional agent unit.

Such covalent bond is indicated in Formula 7 below.

Embodiment 7: The compound according to any one of embodiments 3 to 5, wherein said Linker-Functional agent unit has a structure according to the formula



(Formula 7),

wherein

Z–O'–Sugar is an Iduronide unit ("W"),

wherein

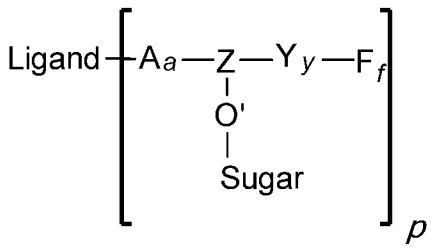
Z is a self-immolative group,

–O'– is a glycosidic bond,

Sugar is a sugar moiety;

A_a–[Z-O'-Sugar]–Y_y is a Linker unit.

Fourth aspect of the present disclosure (also referred to as "Embodiment 8"): According to a fourth aspect, the present disclosure relates to a compound having a structure according to the formula



(Formula 8),

wherein:

Ligand is a Ligand unit;

A is a Stretcher unit;

a is 0, 1 or 2;

Z–O'–Sugar is an Iduronide unit ("W"),

wherein

Z is a self-immolative group,

–O'– is a glycosidic bond,

Sugar is a sugar moiety;

A_a–[Z-O'-Sugar]–Y_y is a Linker unit;

Y is a Spacer unit;

y is 0, 1 or 2;

F is a Functional agent;

f is 1, 2, 3 or 4;

p is from 1 to 20.

As the skilled person understands, within Formula 8, A_a–[Z-O'-Sugar]–Y_y–F_f is a Linker-Functional agent unit.

The definitions and explanations provided below relate to any of the aspects, compounds, methods or uses defined above or below in this disclosure.

Compound

"Compound", as used with respect to compounds according to the present disclosure, is not particularly limited and refers to a chemical entity of any chemical class, provided that it comprises at least one Iduronide unit which is optionally linked to a Stretcher unit and optionally linked to a Spacer unit (and in addition to the different further components specified

for the different aspects and embodiments defined above and below). Thus, the compound can e.g. be a small molecule compound (i.e. a compound with a molecular weight < 1000 Da) or a compound comprising a small-molecule part and a protein part. It can e.g. comprise a protein composed of a single amino acid chain or it can e.g. comprise a protein composed of multiple amino acid chains that are either non-covalently or covalently associated, or a non-covalent complex including an inorganic component. Typically, the compound is a molecule.

Linker unit

Together, the Stretcher unit (A), if present; the Iduronide unit (W); and the Spacer unit (Y), if present; form a Linker unit. The Linker unit is a molecular group that covalently links the Ligand unit and the Functional agent and that has the specific structural features as defined herein. For example, the Linker unit can be a bifunctional compound that links a drug moiety (as Functional agent) to an antibody (as Ligand unit that functions as targeting moiety) to form an ADC compound. The Linker unit according to the present disclosure can be cleaved by enzymatic cleavage with iduronidase enzyme under appropriate conditions (see below and Examples section). The Linker unit according to the present disclosure comprises a self-immolative group. Upon iduronidase-mediated cleavage of a bond within the Linker unit, the self-immolative group spontaneously decomposes such that the covalent link between the Ligand unit and the Functional agent formed by the Linker unit is disrupted and the Functional agent is released.

A Linker unit in a compound according to the present disclosure is preferably stable extracellularly (i.e. outside of the cell, e.g. in plasma). Thus, before transport or delivery into a cell, the compound is preferably stable and remains intact, i.e. the Ligand unit remains linked to the Functional agent. An effective Linker unit will: (i) not interfere with the specific binding properties of the Ligand unit; (ii) allow intracellular delivery of the Functional agent; (iii) remain stable and intact, i.e. not cleaved, until the conjugate has been delivered or transported to its target site; and (iv) maintain the functional efficacy of the Functional agent (e.g. in case of a cytotoxic agent as Functional agent: the cytotoxic, cell-killing effect of the cytotoxic agent).

Methods related to the preparation of linkers in general can be applied to the preparation and linkage of the Linker unit according to the present disclosure. Such method are e.g. described in WO 2004/010957. Further general information about different types of linkers is also e.g. available in Dubowchik and Walker, Pharm. Therapeutics (1999), vol. 83, p. 67-123; U.S.

Patent 6,214,345; U.S. Patent 6,214,345; U.S. Patent 5,122,368; U.S. Patent 5,824,805; U.S. Patent 5,622,929; Neville et al., Biol. Chem. (1989), vol. 264, p. 14653-14661; Thorpe et al., Cancer Res. (1987), vol. 47, p. 5924-5931; Wawrzynczak et al., in: Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (1987), editor Vogel, Oxford U. Press; U.S. Patent 4,880,935; Johnson et al., Anticancer Res. (1995), vol. 15, p. 1387-1393; Lau et al., Bioorg-Med-Chem. (1995), vol. 3(10), p. 1299-1304; Lau et al., Bioorg-Med-Chem. (1995), vol. 3(10), p. 1305-1312.

Covalent linking of the Ligand and the Functional agent via a Linker unit can for example be achieved by a Linker unit having two reactive functional groups (i.e. a linker that is bivalent in a reactive sense). Bivalent linker reagents which are useful to attach two or more functional or biologically active components are known to the skilled person (see e.g. Hermanson, Bioconjugate Techniques (1996), Academic Press (New York), p 234-242).

Alternatively, a Linker-Functional agent unit comprising Functional agent(s) covalently attached to a Linker unit linker may be prepared by methods of organic synthesis. In the case of an ADC, the Linker-Functional agent unit would be a linker-payload construct. Subsequently, one or more copies of this Linker-Functional agent unit can then be conjugated to the Ligand unit by methods known to the skilled person (see e.g. Behrens et al., Molecular Pharmaceutics (2015), vol. 12(11), p. 3986-3998; Stefano, Methods in Molecular Biology (2013), vol. 1045, p. 145-171; Dickgiesser et al., in: Methods in Molecular Biology: Enzyme-Mediated Ligation Methods (2019), editors Nuijens and Schmidt, vol. 2012, p. 135–149; Dickgiesser et al., Bioconjugate Chem. (2020), vol. 31(4), p. 1070-1076) and described in the Examples section below.

A Linker unit according to the present disclosure is particularly preferred if the Functional agent is a therapeutic agent.

For example, in a typical embodiment, the compound according to the present disclosure is an ADC, the Ligand unit is an antibody component (which functions as targeting antibody of the ADC) and the Functional agent is a drug payload (e.g. a cytotoxic agent like exatecan or duocarmycin). In this case, the Linker unit functions as linker that covalently links the targeting antibody to the drug payload of the ADC. Once the ADC comes into contact with iduronidase, the Linker unit is cleaved and the drug payload is released.

Preferably, the Linker unit is stable in the extracellular space of the human body and in the intracellular environment of cells that do not express human α-L-iduronidase.

Preferably, the Linker unit is cleavable in the intracellular environment of cells that express human α-L-iduronidase, such that cleavage of the Linker unit releases the Functional agent (e.g. a drug payload) from the antibody component in the intracellular environment of cells that express human α-L-iduronidase.

Preferably, the target cell (i.e. the cell type to which the Ligand unit recruits the compound of the present disclosure) is a cell that expresses iduronidase enzyme in its cytoplasm.

As the skilled person understands, in compounds according to the present disclosure comprising a Ligand, a Linker unit and a Functional agent this allows for release of the Functional agent (which initially is covalently attached to the Ligand via the Linker unit) into the target cells that express human α-L-iduronidase.

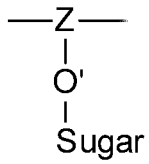
Typically, there will be one Functional agent per Linker unit, i.e. one Linker unit molecule for each individual occurrence of a Functional agent in the compound (in the case of an ADC, one linker per payload). This means that, if e.g. two copies of a Functional agent are present in an ADC, there will usually be two Linker units, wherein the first Linker unit covalently links the first Functional agent to the Ligand, and the second Linker unit covalently links the second Functional agent to the Ligand. However, in certain embodiments it is also possible that one Linker unit links more than one Functional agent to the Ligand of the ADC (e.g. in cases where $f > 1$).

Iduronide unit

The Iduronide unit (W) is a component of the Linker unit that links a Stretcher unit to a Spacer unit if Stretcher and Spacer units are present in said compound, links a Stretcher unit to the Functional agent (such as the drug payload of an ADC or a fluorophore), if a Stretcher unit is present and no Spacer unit is present in said compound, links the Ligand unit (such as the antibody component of an ADC) to a Spacer unit, if no Stretcher unit is present and a Spacer unit is present in said compound, and links the Ligand unit to the Functional agent if neither a Stretcher unit nor a Spacer unit is present in said compound.

The Iduronide unit comprises an iduronidase cleavage site.

Preferably, the Iduronide unit (W) comprises (preferably is) a sugar moiety ("Sugar") linked via a glycosidic bond (–O'–) to a self-immolative group (Z), according to the formula:



(Formula 9).

The glycosidic bond (–O'–) is an iduronidase cleavage site, preferably a bond cleavable by α-L-iduronidase, more preferably a bond cleavable by human α-L-iduronidase.

In general terms, an iduronidase is an enzyme that catalyzes the hydrolysis of iduronosidic linkages.

α-L-iduronidase is, more specifically, an enzyme that catalyzes the hydrolysis of unsulfated α-L-iduronosidic linkages (enzyme classification number E.C. 3.2.1.76). The systematic name of the enzyme is glycosaminoglycan α-L-iduronohydrolase. In nature, α-L-iduronidase is a glycoprotein enzyme found in the lysosomes of cells. It is involved in the degeneration of glycosaminoglycans such as dermatan sulfate and heparan sulfate. The enzyme acts by hydrolyzing the terminal α-L-iduronic acid residues of these molecules, thereby degrading them.

As used herein in the context of an Iduronide unit, the term "self-immolative group" refers to a di- or tri-functional chemical moiety that is capable of covalently linking together two or three spaced chemical moieties into a stable molecule and that, upon cleavage of a certain bond, decomposes in such a way that the covalent linkage between said two (or three) chemical moieties is broken and the individual chemical moieties are released. In the present case, the self-immolative group may for example provide a covalent linkage between the sugar moiety (via a glycosidic bond), a Functional agent (e.g. a drug moiety; either directly or indirectly via a Spacer unit), and a Ligand unit (either directly or indirectly via a Stretcher unit). Once the

glycosidic bond between the self-immolative group and the iduronide sugar is cleaved by enzymatic cleavage with iduronidase, the self-immolative group degrades in such a manner that also the linkage between the Functional agent and the Ligand unit is severed and the Functional agent is released.

The sugar moiety is an iduronide moiety (i.e. an α-L-iduronic acid or β-L-iduronic acid linked to the self-immolative group Z via a glycosidic bond that is cleavable by iduronidase).

Preferably, the sugar moiety is an α-L-iduronide moiety (i.e., α-L-iduronic acid linked to the self-immolative group Z via a glycosidic bond that is cleavable by α-L-iduronidase).

Stretcher unit

The Stretcher unit (A), when present in the compound of the present disclosure, is a molecular group that is covalently linked to the Ligand unit and the Iduronide unit (W) and thus forms a connection between these two molecular components of the compound according to the present disclosure. If the compound of the present disclosure comprises a Ligand unit, but no Stretcher unit, then the Ligand unit is linked to the Iduronide unit directly via a covalent bond.

With respect to its chemical structure, the Stretcher unit is not particularly limited, provided that it forms a covalent connection between the Ligand unit and the Iduronide unit and does not interfere with the function of the compound according to the present disclosure (in particular with the binding/targeting function of the Ligand unit, the iduronidase-mediated cleavage of the Iduronide unit and the activity of the Functional agent).

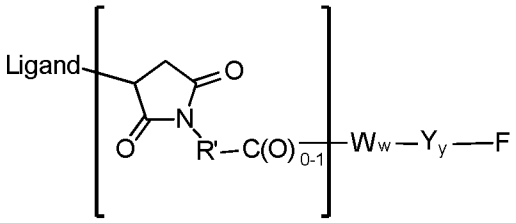
Formation of a covalent linkage between the Ligand unit and the Stretcher unit can for example be achieved through a functional group of the Ligand unit that allows to form a bond with a functional group of the Stretcher unit. Useful functional groups that can be present on a Ligand unit (either naturally or upon introduction via chemical manipulation) include, but are not limited to, sulfhydryl (–SH), amino, hydroxyl, carboxy, the anomeric hydroxyl group of a carbohydrate, and carboxyl.

Preferably, the functional groups of the Ligand unit are sulfhydryl and/or amino groups. Sulfhydryl groups can be generated by reduction of an intramolecular disulfide bond of a Ligand. Sulfhydryl groups also can be generated by reaction of an amino group of a lysine

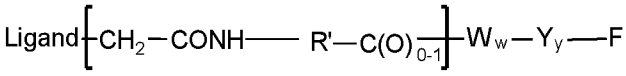
moiety of a Ligand unit using 2-iminothiolane (Traut's reagent) or another sulfhydryl generating reagent.

The Stretcher unit may form a bond with a sulfur atom of the Ligand unit. The sulfur atom for this bond formation can be derived from a sulfhydryl group of the Ligand unit.

Exemplary Stretcher units can be e.g. of the general format depicted within the square brackets of the following two formulas:



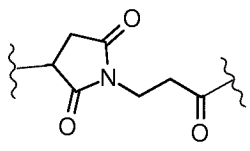
(Formula 10)



(Formula 11),

wherein R' is a direct bond or selected from C_1-C_{10} alkylene, C_3-C_8 carbocyclo, –O–(C_1-C_8 alkyl), arylene, C_1-C_{10} alkylene-arylene-, arylene-C_1-C_{10} alkylene, C_1-C_{10} alkylene (C_3-C_8 carbocyclo), (C_3-C_8 carbocyclo) –C_1-C_{10} alkylene-, C_3-C_8 heterocyclo-, –C_1-C_{10} alkylene-(C_3-C_8 heterocyclo)-, (C_3-C_8 heterocyclo) –C_1-C_{10} alkylene, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, and –$(CH_2CH_2O)_r$–CH_2–CH_2–; and r is an integer ranging from 1-10.

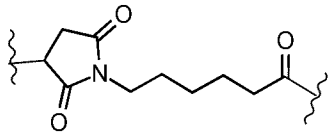
A specific example of a Stretcher unit according to the present disclosure is a Stretcher unit according the following formula:



(Formula 12).

As the skilled person understands, this corresponds to Formula 10, wherein R' is —$(CH_2)_2$—.

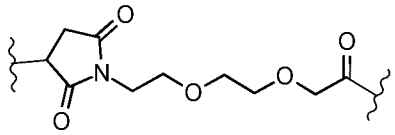
Another specific example of a Stretcher unit according to the present disclosure is a Stretcher unit according to the following formula:



(Formula 13).

As the skilled person understands, this corresponds to Formula 10, wherein R' is —$(CH_2)_5$—.

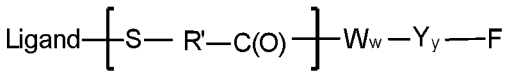
A further specific example of a Stretcher unit according to the present disclosure is a Stretcher unit according to the following formula:



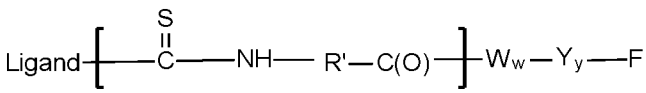
(Formula 14).

As the skilled person understands, this corresponds to Formula 10, wherein R' is—$(CH_2CH_2O)_r$—CH_2—; and r is 2.

Alternatively, the Stretcher unit may be linked to the Ligand unit via a disulfide bond between a sulfur atom of the Ligand unit and a sulfur atom of the Stretcher unit. A representative Stretcher unit of this embodiment is depicted within the square brackets of the following formulas:



(Formula 15)



(Formula 16),

wherein R' is a direct bond or selected from C_1-C_{10} alkylene, C_3-C_8 carbocyclo, –O–(C_1-C_8 alkyl), arylene, C_1-C_{10} alkylene-arylene-, arylene-C_1-C_{10} alkylene, C_1-C_{10} alkylene (C_3-C_8 carbocyclo), (C_3-C_8 carbocyclo) –C_1-C_{10} alkylene-, C_3-C_8 heterocyclo-, –C_1-C_{10} alkylene-(C_3-C_8 heterocyclo)-, (C_3-C_8 heterocyclo) –C_1-C_{10} alkylene, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, and –$(CH_2CH_2O)_r$–CH_2–CH_2–; and r is an integer ranging from 1-10.

Within a Linker unit (resp. within a Linker-Functional agent unit), there may be one Stretcher unit A or more than one Stretcher unit A (as defined by the parameter *a*, wherein $a > 1$ indicates the presence of several Stretcher units A). At each such occurrence of the Stretcher unit, the structure of the Stretcher unit A is independently selected within the structural limits defined herein.

Moreover, there may be more than one Linker-Functional agent unit in a compound according to the present disclosure (as defined by the parameter *p*), which can also result in a situation where there are multiple Stretcher units present in a compound according to the present disclosure. If there is more than one Stretcher unit present, these multiple Stretcher units may all have an identical structure or may not have an identical structure (within the limits set by the structural features as defined above or below).

Preferably, if a Stretcher unit is present, there is only one Stretcher unit per Linker-Functional agent unit (i.e. that Stretcher unit is present in a single copy in said Linker-Functional agent unit, i.e. *a* is 1).

Preferably, all Stretcher units within a compound according to the present disclosure have the same chemical structure.

Spacer unit

The Spacer unit (Y), when present in the compound of the present disclosure, is a molecular group that is covalently linked to the Iduronide unit (W) and the Functional agent and thus forms a connection between these two molecular components of the compound according to the present disclosure. If the compound of the present disclosure comprises a Functional agent, but no Spacer unit, then the Iduronide unit is linked to the Functional agent directly via a covalent bond.

The Spacer unit(s) may be a self-immolative spacer.

In this context, the term "self-immolative spacer" refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a normally stable tripartite molecule. It will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved.

With respect to its chemical structure, the Spacer unit is not particularly limited, provided that it forms a covalent connection between the Iduronide unit and the Functional agent and does not interfere with the function of the compound according to the present disclosure (in particular with the binding/targeting function of the Ligand unit, the iduronidase-mediated cleavage of the Iduronide unit and the activity of the Functional agent).

The Spacer unit may be linked to the Iduronide unit e.g. via the methylene carbon atom of the self-immolative group, and linked directly to the Functional agent via a carbonate, carbamate or ether group.

Without being bound by any particular theory or mechanism, Figure 4A depicts a mechanism of release of a Functional agent (in this case the drug exatecan) of an iduronide-based linker which is attached directly to a Functional agent (in this case the drug exatecan) via a carbamate group.

In one example, said Spacer unit is a meta-aminobenzyl alcohol unit whose phenylene portion is substituted with Q_m wherein Q is —C1-C8 alkyl, -O-(C1-C8 alkyl), -halogen, -nitro or -cyano; and *m* is an integer ranging from 0-4. In another embodiment, -Yy- can be a carbonate group.

Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the meta-aminobenzyl alcohol group such as 2-aminoimidazol-5- methanol derivatives (see, e.g., Hay et al., 1999, Bioorg. Med. Chem. Lett. 9:2237) and ortho or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (see, e.g., Rodrigues et al., 1995, Chemistry Biology 2:223), appropriately substituted bicyclo 2.2.1 and bicyclo2.2.2 ring systems (see, e.g., Storm et al., 1972, J. Amer: Chem. Soc. 94:5815) and 2-aminophenylpropionic acid amides (see, e.g., Amsberry et al., 1990, J. Org. Chem. 55:5867). Elimination of amine-containing drugs that are substituted at the a-position of glycine (see, e.g., Kingsbury et al., 1984, J. Med. Chem. 27:1447) are also examples of self-immolative spacers.

Within a Linker-Functional agent unit, there may be one Spacer unit Y or more than one Spacer unit Y (as defined by the parameter *y*, wherein $y > 1$ indicates the presence of several Spacer

units Y). At each such occurrence of the Spacer unit, the structure of the Spacer unit Y is independently selected within the structural limits defined herein.

Moreover, there may be more than one Linker-Functional agent unit present in a compound according to the present disclosure (as defined by the parameter *p*), which can also result in a situation where there are multiple Spacer units present in a compound according to the present disclosure. If there is more than one Spacer unit present, these multiple Spacer units may all have an identical structure or may not have an identical structure (within the limits set by the structural features as defined above or below).

Preferably, if a Spacer unit is present, there is only one Spacer unit per Linker-Functional agent unit (i.e. that Spacer unit is present in a single copy in said Linker-Functional agent unit, i.e. *y* is 1).

Preferably, all Spacer units within a compound according to the present disclosure have the same chemical structure.

Ligand unit

The Ligand unit is a moiety (i.e. a molecular group or chemical structure) that is capable of directing the compound of the present disclosure to a target site, such as a binding site on a target cell. The target site will typically be a biological molecule or a certain part of a biological molecule. The Ligand unit binds to said biological molecule or part of a biological molecule, and through said binding directs the compound comprising said Ligand unit to said binding site. For example, the Ligand unit may be a moiety that specifically binds to EGFR and thus targets the compound to cells expressing EGFR at their cell surface.

An example of a Ligand unit is an antigen-binding antibody fragment that is covalently linked via an Iduronide unit to a drug payload (i.e. a Functional agent) to form an ADC (i.e. a compound according to the present disclosure), wherein the antigen-binding fragment binds to a certain receptor present at the surface of a certain cell type (its antigen); binding of the antigen-binding fragment to this receptor results in recruitment of the compound to this cell (i.e. the antigen-binding fragment acts as targeting moiety).

Non-targeted compounds such as non-targeted drugs typically reach their site of action by whole-body distribution and passive diffusion. In contrast, targeted compounds are not distributed evenly across the whole body. Due to the interaction of targeting moiety with its target molecule, a compound including a targeting moiety is concentrated preferentially at its site target site. Therefore, e.g. therapeutic compounds with a targeting moiety (e.g. a Ligand unit as described herein) require lower dosages to be therapeutically effective, thus improving the therapeutic window.

The Ligand unit is covalently linked to the Linker unit. Specifically, the Ligand unit is covalently linked to the Iduronide unit, either directly or indirectly via a Stretcher unit, if present (see e.g. in Formula 5 above).

With respect to its structure, the Ligand unit is not particularly limited, provided that it specifically binds to a desired binding site in such a manner that it can recruit the compound of the present disclosure to the desired target site.

The Ligand unit may for example be a protein, such as a protein ligand of a receptor on the target cell. Thus, upon binding of said protein ligand to its receptor on the cell surface of said target cell, the compound of the present disclosure (of which said protein ligand is a part) is recruited to the surface of said target cell. Alternatively, the Ligand unit may for example be a peptide, a peptide mimetic, a nucleic acid, an oligonucleotide or a small molecule. As used herein, a small molecule is a molecule with a molecular weight < 1 000 Da.

Alternatively, the Ligand unit may be (or comprises) an antibody or an antigen-binding fragment of an antibody. For example, the Ligand unit may be an antibody, which specifically binds to an antigen that is present on the surface of a target cell and thus recruits the compound of the present disclosure (of which that Ligand unit is a part) to said target cell.

An "antibody" is a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or antigen binding fragment thereof, which specifically binds and recognizes an analyte (antigen). Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes.

Preferably, the term "antibody", as used herein, refers to an immunoglobulin molecule that is used or can be used as part of a compound according to the present disclosure, e.g. an immunoglobulin molecule that is used or can be used as part of an antibody-drug conjugate. The term "antibody" can encompass full-length antibodies and antigen-binding fragments of full-length antibodies (i.e. fragments of an full-length antibody that are still capable of binding the same antigen to which the corresponding full-length antibody binds). In some embodiments, the term also includes molecules in which a full-length antibody or antigen-binding fragment of a full-length antibody is covalently linked to one or more further full-length antibodies and/or one or more further antigen-binding fragments of antibodies and/or another molecular structure. Thus, the antibody is an immunoglobulin molecule that recognizes and specifically binds to a target (the antigen, see below), through at least one antigen-binding site within the variable region of the immunoglobulin molecule.

In primates such as humans, a heavy and the light chain variable domain of an antibody combine to specifically bind the antigen. Generally, a naturally occurring primate (e.g., human) or murine immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Primate antibodies can be class switched.

The antibody according to the present disclosure can be of any class (e.g. IgA, IgD, IgE, IgG, and IgM, preferably IgG), or subclass (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2, preferably IgG1). The different classes of immunoglobulins have different and well-known subunit structures and three-dimensional configurations (Immunobiology, 5th ed. (2001), editors Janeway et al., Garland Publishing (USA)).

Specifically, each light and heavy chain of an antibody contains constant domains and variable domains. References to "VH" or "VH" refer to the variable region of an immunoglobulin heavy chain, including that of an antibody fragment. References to "VL" or "VL" refer to the variable region of an immunoglobulin light chain, such as in a primate antibody.

Light and heavy chain variable domains contain a "framework" region interrupted by three hypervariable regions, also called "complementarity determining regions" or "CDRs" (see, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Department of Health and

Human Services, 1991). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space. The CDRs are primarily responsible for antigen binding.

The CDRs are typically referred to as CDR1, CDR2, and CDR3 (from the N-terminus to C-terminus), and are also typically identified by the chain in which the particular CDR is located. Thus, a VH CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a VL CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. Light chain CDRs are sometimes referred to as CDR L1, CDR L2, and CDR L3. Heavy chain CDRs are sometimes referred to as CDR H1, CDR H2, and CDR H3. VHH monoclonal antibodies have only a heavy chain, and thus include only one CDR1, CDR2 and CDR3. Generally, the CDR3 is primarily responsible for antigen specificity. The extent of the framework region and CDRs have been defined (see, Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1991). The Kabat database is now maintained online.

The antibody that is included as Ligand unit in the compound according to the present disclosure may for example be an antibody selected from the group consisting of an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, IgG4 antibody, an IgA antibody, an IgM antibody, and hybrids thereof.

An antibody consisting of a "hybrid" of two antibodies of different class/subclasses refers to an antibody that contains sequences from these two antibodies of different class/subclass. For example, a bispecific antibody prepared by the SEED technology (WO 2016/087650) typically contains sequences from both IgG and IgA and thus would be considered a "hybrid" of an IgG antibody and an IgA antibody.

As used herein, a "full-length" antibody refers to an antibody that includes the complete, full-length sequence of an antibody of the respective antibody class. Thus, a full-length antibody includes the antigen-binding region(s) (i.e. the complete VL and VH domains), as well as complete light and heavy chain constant domains, as appropriate for the antibody class, wherein the antibody domains remain associated through at least one non-covalent interaction. The

constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof.

As used herein, a "fragment" of an antibody is a portion of a full-length antibody. An "antigen-binding fragment" of a (full-length) antibody is a portion of said antibody that binds the same antigen as the full-length antibody. For example if a full-length antibody is directed against and binds to cMET, an antigen-binding fragment of said antibody also binds to cMET. Thus, said antigen-binding fragment binds to the same antigen as the full-length antibody from which said fragment is derived (namely, in this example, cMET). Typically, this means that the fragment comprises the same antigen-binding region as the corresponding full-length antibody. Examples of antibody fragments include, but are not limited to Fab, Fab', F(ab')2, and Fv fragments and single chain Fv (scFv) antibodies. In some embodiments, the term "fragment" of an antibody also encompasses bi- or multivalent antibody constructs generated by joining two or more of the aforementioned antibody fragments together.

As used herein, "antigen" refers to a substance that can specifically bind to the variable region of an antibody. An antigen may e.g. be a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or a combination of the foregoing.

A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of the antibody. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (Sequences of Proteins of Immunological Interest, 5th ed. (1991), editors Kabat et al., National Institutes of Health (Bethesda, USA)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-Lazikani et al., J. Molec. Biol. (1997), vol. 273, p. 927-948)). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

The terms "epitope" or "antigenic determinant" are used interchangeably herein and refer to the portion of an antigen that is recognized and specifically bound by a particular antibody. When

the antigen is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

Standard techniques of antibody design and preparation are known to a skilled person (see e.g. Antibodies: A Laboratory Manual, 2nd edition (2014), editor Greenfield, Cold Spring Harbor Laboratory Press (U.S.); Antibody Engineering - Methods and Protocols, 2nd edition (2010), editors Nevoltris and Chames, publisher Springer (Germany); Handbook of Therapeutic Antibodies (2014), editors Dübel and Reichert, publisher Wiley-VCH Verlag GmbH & Co. KGaA (Germany); Harper, Methods in Molecular Biology (2013), vol. 1045, p. 41-49).

With respect to the type and source of the antibody, the antibody according to the present disclosure (i.e. the antibody that is included as Ligand unit in the compound according to the present disclosure) is not particularly limited, as long as it contains at least one antigen-binding site and shows binding to its target antigen.

Thus, the antibody that is included as Ligand unit in the compound according to the present disclosure may not only be a "classical" full-length antibody or fragment thereof, but can e.g. also be a Fab, a Fab', a (Fab')2, a Fv, a scFv, a diabody or a VHH.

"Fab" fragments are obtained by papain digestion of an antibody, which produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (CH1). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site.

"F(ab')2" fragments are obtained by pepsin treatment of an antibody, which yields a single large F(ab')2 fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen.

"Fab' " fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')2 antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv", also abbreviated as "scFv", are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of the scFv, see Pluckthun, in: The Pharmacology of Monoclonal Antibodies, vol. 113 (1994), editors Rosenburg and Moore, Springer-Verlag (New York), p. 269-315.

The term "diabody" refers to a small antibody fragment prepared by constructing scFv fragments (see preceding paragraph) with short linkers (about 5-10) residues) between the VH and VL domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, i.e., a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" scFv fragments in which the VH and VL domains of the two antibodies are present on different polypeptide chains. Diabodies are

described in greater detail in, for example, EP 0404097; WO 93/11161; Hollinger et al., Proc. Natl. Acad. Sci. USA (1993), vol. 90, p. 6444-6448.

As used herein, the terms "VHH" and "nanobody" have the same meaning. They refer to single-domain antibodies which are antibody fragments consisting of a single monomeric variable region of a heavy chain of an antibody. Like a whole antibody, a VHH is able to bind selectively to a specific antigen. With a molecular weight of only 12–15 kDa, VHHs are much smaller than common antibodies (150–160 kDa). The first single-domain antibodies were engineered from heavy-chain antibodies found in camelids. (Gibbs and Wayt, Nanobodies, Scientific American Magazine (2005)). Generally, the antibodies with a natural deficiency of the light chain and the heavy chain constant region 1 (CH1) are first obtained, the variable regions of the heavy chain of the antibody are therefore cloned to construct a single domain antibody (VHH) consisting of only one heavy chain variable region.

The antibody that is included as Ligand unit in the compound according to the present disclosure may be a monovalent, bivalent or multivalent antibody, it may be a monospecific or a bispecific antibody, and/or it may be a chimeric, a humanized or a human antibody.

A "monospecific antibody", as used herein, is an antibody that is capable of binding only to one antigen.

The term "bispecific antibody", as used in the present disclosure, refers to an antibody that is capable of specifically binding to two different epitopes at the same time. The epitopes can be from the same antigen or from two different antigens. Preferably, the epitopes are from two different antigens. Typically, a bispecific antibody has two antigen-binding sites, wherein e.g. each of the two pairs of heavy chain and light chain (HC/LC) is specifically binding to a different antigen, i.e. the first heavy and the first light chain are specifically binding together to a first antigen, and, the second heavy and the second light chain are specifically binding together to a second antigen. Methods for making bispecific antibodies are known in the art. For example, bispecific antibodies can be produced recombinantly using the co-expression of two immunoglobulin heavy chain/light chain pairs (see e.g. Milstein et al., Nature (1983), vol. 305, p. 537-539). Alternatively, bispecific antibodies can be prepared using chemical linkage (see e.g. Brennan et al., Science (1985), vol. 229, p. 81). A bispecific antibody can also for example be prepared by the SEED technology (an approach for generation of bispecific antibodies in

which structurally related sequences within the conserved CH3 domains of human IgA and IgG are exchanged to form two asymmetric but complementary domains, see WO 2016/087650).

A "monovalent" antibody has one antigen-binding site. A "bivalent" antibody has two antigen-binding sites. These two antigen-binding sites may bind the same or different antigens. A "multivalent" antibody has more than two antigen-binding sites. These more than two antigen-binding sites may bind the same or different antigens.

As used in this disclosure, a "chimeric" antibody is an antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent 4,816,567; Morrison et al., Proc. Natl. Acad. Sci USA (1984), vol. 81, p. 6851-6855). As used herein, "humanized antibody" is used a subset of "chimeric antibodies."

A "humanized antibody", as used herein, is a "humanized" form of non-human (e.g., murine) antibody. A "humanized antibody", is a chimeric antibody that contains minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR (hereinafter defined) of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, etc. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no more

than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see e.g. Jones et al., Nature (1986), vol. 321, p. 522-525; Riechmann et al., Nature (1988), vol. 332, p. 323-329; and Presta, Curr. Op. Struct. Biol. (1992), vol. 2, p. 593-596. See also, for example, Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. (1998), vol. 1, p. 105-115; Harris, Biochem. Soc. Transactions (1995), vol. 23, p. 1035-1038; Hurle and Gross, Curr. Op. Biotech. (1994), vol. 5, p. 428-433; U.S. Patent 6,982,321; U.S. Patent 7,087,409.

A "human antibody" is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries (Hoogenboom and Winter, J. Mol. Biol. (1991), vol. 227, p. 381; Marks et al., J. Mol. Biol. (1991), vol. 222, p. 581). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., in: Monoclonal Antibodies and Cancer Therapy (1985), editors Reisfeld and Sell, publisher Alan R. Liss Inc. (New York), p. 77-96; Boerner et al., J. Immunol. (1991), vol. 147(1), p. 86-95; van Dijk and van de Winkel, Curr. Opin. Pharmacol. (2001), vol. 5, p. 368-374. Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e.g., immunized xenomice (see e.g. U.S. Patent 6,075,181 and U.S. Patent 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li et al., Proc. Natl. Acad. Sci. USA (2006), vol. 103, p. 3557-3562 regarding human antibodies generated via a human B-cell hybridoma technology.

The antibody that is included as Ligand unit in the compound according to the present disclosure may be a monoclonal antibody or a polyclonal antibody.

A "monoclonal" antibody", as used herein, means an antibody arising from a nearly homogeneous antibody population. More particularly, the individual antibodies of a population are identical except for a few possible naturally-occurring mutations which can be found in minimal proportions. In other words, a monoclonal antibody consists of a homogeneous antibody arising from the growth of a single cell clone and is generally characterized by heavy chains of one (i.e. only one) class and subclass, and light chains of only one type. Monoclonal

antibodies are directed against a single antigen. In addition, in contrast with preparations of polyclonal antibodies which typically include various antibodies directed against various epitopes, each monoclonal antibody is directed against a single epitope of the antigen. Monoclonal antibodies are typically produced by a single clone of B lymphocytes ("B cells"). Monoclonal antibodies may be obtained using a variety of techniques known to those skilled in the art, including standard hybridoma technology (see e.g. Köhler and Milstein, Eur. J. Immunol. (1976), vol. 5, p. 511-519; Antibodies: A Laboratory Manual, 2nd edition (2014), editor Greenfield, Cold Spring Harbor Laboratory Press (USA); Immunobiology, 5th ed. (2001), editors Janeway et al., Garland Publishing (USA)) and e.g. expression from a eukaryotic host cell transfected with a DNA molecule coding for the homogeneous antibody or from a prokaryotic host cell transfected with a DNA molecule coding for the homogeneous antibody.

As used herein, "polyclonal" antibody refers to a heterogeneous population of antibodies, typically obtained by purification from the sera of immunized animals by standard techniques known to a skilled person (see e.g. Antibodies: A Laboratory Manual, 2nd edition (2014), editor Greenfield, Cold Spring Harbor Laboratory Press (USA)).

Typically, each compound according to the present disclosure comprises one antibody (but may comprise e.g. more than one Linker unit and more than one Functional agent).

In some embodiments, the Ligand unit comprises additional molecular group(s) in addition to the part of the molecule that is directly involved in the binding to the target site.

In some embodiments, the Ligand unit does not comprise additional molecular groups in addition to the part of the molecule that is directly involved in the binding to the target site.

Functional agent

The Functional agent is a chemical entity which is capable of fulfilling a biological, chemical, therapeutic and/or diagnostic function in the human body. The terms "Functional agent" and "payload" are used synonymously herein.

As the skilled person understands, the term "chemical entity" includes any type of chemical group or molecule of any substance class and is only limited by the recited requirement that it

must be capable of fulfilling (in the context of the compound according to the present disclosure) a biological, chemical, therapeutic and/or diagnostic function in the human body.

For example, the Functional agent can be a therapeutic agent or a detectable label.

A "therapeutic agent", as used herein, is an agent that exerts an effect that is linked to a therapeutic benefit if administered to a patient (e.g. by killing a tumor cells, reducing an undesired inflammation, stimulating the activity of the immune system against an infection, or suppressing the immune response in case of an autoimmune disease). The terms "therapeutic agent" and "drug moiety" (D) are used synonymously herein.

Different therapeutic agents, their preparation, conjugation and use in compounds like antibody-drug conjugates are described e.g. in Nicolaou and Rigol, 2019; Maderna and Leverett, 2015; Gromek and Balunas, 2014.

Therapeutic agents useful in accordance with the present disclosure include, but are not limited to, cytotoxic agents, anti-inflammatory agents, immunostimulatory agents and immunosuppressive agents.

Thus, the therapeutic agent may be a cytotoxic agent.

As used herein, a "cytotoxic agent" is a substance that is toxic to cells (i.e. causes cell death or destruction). A cytotoxic agent according to the present disclosure is typically a small molecule chemical compound, peptide, or nucleic acid molecule. Various cytotoxic agents that can be used in compounds according to the present disclosure (such as ADCs) are known to the skilled person (Nicolaou and Rigol, 2019; Maderna and Leverett, 2015; Gromek and Balunas, 2014; Garcia-Echeverria, 2014).

Cytotoxic agents may achieve cell killing by different mechanisms and thus divided into different classes according to their mechanism of action (Nicolaou and Rigol, 2019).

In some embodiments, the cytotoxic agent included in the compound of the present disclosure is selected from the group consisting of an inhibitor of microtubule formation, an EG5 inhibitor and a DNA damaging agent (e.g. Anderl et al., 2013).

An "inhibitor of microtubule formation", as used herein, is an inhibitor that acts by inhibiting tubulin polymerization or microtubule assembly, and thus has anti-proliferative/toxic effects on cells. For example, said inhibitor of microtubule formation may be an auristatin, a maytansinoid or tubulysin.

An "EG5 inhibitor", as used herein, is an inhibitor that inhibits the protein EG5, and thus is toxic to cells. EG5 refers to member 11 of the human kinesin family, which is also known as KIF11, HKSP, KNSL1 or TRIP5. EG5 inhibitors are for example those described in Marconi et al., 2019 or Karpov et al., 2019. For example, said EG5 inhibitor may be ispinesib, filanesib, litronesib or K858 (Chen et al., 2017).

A "DNA damaging agent", as used herein, is an agent that acts to damage cellular DNA, e.g. by inducing double-strand breaks, cross-linking specific sites of DNA or intercalating between DNA base pairs. Said DNA damaging agent may for example be a topoisomerase I inhibitor (e.g. exatecan, or camptothecin), a topoisomerase II inhibitor (e.g. doxorubicin or a variant thereof) or a DNA alkylating agent (e.g. a duocarmycin, a CBI dimer, a pyrrolobenzodiazepine or a variant thereof).

Therapeutic agents that can be used as Functional agent in the compound according to the present disclosure include all cytotoxins commonly utilized in ADCs to date. Most classes of cytotoxins act to inhibit cell division and are classified based on their mechanism of action. Exemplary cytotoxins that are conceivable as part of the inventive conjugates include, without limitation, anthracycline, doxorubicin, methotrexate, auristatins including monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), maytansines and their maytansinoid derivatives (DMs), calicheamicins, duocarmycins and pyrrolobenzodiazepine (PBD) dimers, topoisomerase I inhibitors, e.g. camptothecins and camptothecin derivates, triptolides.

The therapeutic agent may for example be a topoisomerase I inhibitor (e.g. exatecan, camptothecin, SN38, Dxd or a variant thereof), a topoisomerase II inhibitor (e.g. doxorubicin or a variant thereof), a DNA alkylating agent (e.g. duocarmycin, a CBI dimer, a pyrrolobenzodiazepine or a variant thereof), a transcription inhibitor (e.g. triptolide, CDK inhibitors), a Bcl-xL inhibitor (e.g. clezutoclax), a tyrosine kinase inhibitor (e.g. neolymphostin,

dasatinib or staurosporine), an immune-stimulating agent (e.g. a STING or TLR agonist), an HSP90 inhibitor (e.g. a geldanamycin derivate), a splicing inhibitor (e.g. a pladienolide), a translation inhibitor (e.g. psymberin), a proteasome inhibitor (e.g. a carmaphycin B analogue) or a PROTAC (e.g GNE-987).

In some embodiments, the therapeutic agent is selected from the group consisting of a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, an amanitin, a pyrrolobenzodiazepine, an RNA polymerase inhibitor, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor. In some embodiments, the cytotoxic agent is a maytansinoid, wherein the maytansinoid is N(2')-deacetyl-N(2')-(3-mercapto-l-oxopropyl)-maytansine (DM1), N(2')-deacetyl-N(2')-(4-mercapto-l-oxopentyl)-maytansine (DM3) and N(2')-deacetyl-N2-(4- mercapto-4-methyl- 1 -oxopentyl)-maytansine (DM4).

Preferably, the cytotoxic agent is selected from the group consisting of: anthracycline, doxorubicin, methotrexate, an auristatin including monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), maytansines and their maytansinoids derivatives (DMs), calicheamicins, duocarymycins and pyrrolobenzodiazepine (PBD) dimers, a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an amanitin, a pyrrolobenzodiazepine, an RNA polymerase inhibitor, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

In a particular preferred embodiment, the drug is the tubulin inhibitor monomethyl auristatin E (MMAE).

Methods for attaching each of these to a compound as according to the present disclosure are known in the art (see e.g. Singh and Erickson, 2009).

Further details about cytotoxic payloads for ADCs can for example be found in Cytotoxic Payloads for Antibody-Drug Conjugates (Drug Discovery, Band 71), 1st edition (2019), editors Thurston and Jackson, Royal Society of Chemistry (U.K.).

A cytotoxic agent may for example be a radioactive isotope.

Examples for cytotoxic agents that are radioactive isotopes are At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, P^{212}, Zr^{89} and radioactive isotopes of Lu.

In some embodiments, the Functional agent is a cytotoxic agent that shows a higher cytotoxicity after linker cleavage and release compared to the previous state when the cytotoxic agent is still covalently attached via the linker to the compound according to the present disclosure (e.g. to an ADC).

The compound according to the present disclosure may comprise only one type of payload (i.e., if the compound according to the present disclosure is e.g. an antibody-drug conjugate (ADC), one ADC molecule according to the present disclosure is linked to only one kind of payload, e.g. auristatin E, wherein one or more copies of the payload (in this example auristatin E) may be linked to the ADC molecule). Alternatively, the compound according to the present disclosure may comprise several types of payloads (i.e., if the compound according to the present disclosure is e.g. an antibody-drug conjugate (ADC), one ADC molecule is linked to two or more kinds of therapeutic agent, e.g. auristatin E and DM4, wherein one or more copies of each payload (in this example one or more copies of auristatin E and one or more copies of DM4) may be linked to the ADC molecule). Preferably, the compound according to the present disclosure comprises only one kind of payload.

If the compound according to the present disclosure is an ADC, the copy number of Functional agents linked to one compound (i.e. in the first example above the number of auristatin E molecules linked to one ADC molecule, and in the second example above the number of

auristatin E molecules plus the number of DM4 molecules linked to one ADC molecule) is reflected in the drug-antibody ratio (DAR).

Alternatively, the therapeutic agent may be an anti-inflammatory agent.

As used herein, an "anti-inflammatory agent" is a substance that reduces inflammation. This means that said anti-inflammatory agent results in the reduction of an undesired inflammation as compared to the administration of a control molecule that does not include said anti-inflammatory agent. By recruiting the anti-inflammatory agent to specific immune cells as target cells, the anti-inflammatory effects can be focused to the site of inflammation (where these immune cells may be enriched) or to a specific type of immune cell.

The anti-inflammatory agent may be a glucocorticoid receptor agonist. For example, the anti-inflammatory agent may be a steroid, preferably, selected from the group consisting of cortisol, cortisone acetate, beclometasone, prednisone, prednisolone, methylprednisolone, betamethasone, trimcinolone, budesonide, dexamethasone, fluticasone, fluticasone propionate, fluticasone furoate and a mometasone; or the anti-inflammatory agent may be a non-steroidal anti-inflammatory agent, e.g. a Cox2 inhibitor.

Alternatively, the therapeutic agent may be an immunostimulatory agent.

As used herein, an "immunostimulatory agent" is a substance that enhances the development or maintenance of an immunologic response. The immunostimulatory agent may be an agonist of an immunostimulatory molecule or an antagonists of a molecule inhibiting an immunologic response. In some embodiments, the immunostimulatory agent comprises an agonist of an immunostimulatory molecule, such as an agonist of a costimulatory molecule found on immune cells such (as T cells) or an agonist of a costimulatory molecule found on cells involved in innate immunity (such as NK cells). In some embodiments, the immunostimulatory agent comprises an antagonist of an immunosuppressive molecule, e.g. an antagonist of a cosuppressive molecule found on cells involved in innate immunity (such as NK cells). Preferably, administration of an compound with an immunostimulatory agent as payload results in an improvement of a desired immune response. In some embodiments, administration of an ADC with an immunostimulatory agent as payload results in an improved anti-tumor response

in an animal cancer model, such as a xenograft model, as compared to the administration of a control molecule that does not include said immunostimulatory agent.

In some embodiments, the immunostimulatory agent is or comprises an antagonist of an inhibitor of T cell activation. In some embodiments, the immunostimulatory agent is or comprises an agonists of a stimulant of T cell activation. In some embodiments, the immunostimulatory agent is or comprises an agent that antagonizes or prevents cytokines that inhibit T cell activation, such as IL-6, IL-10, TGFβ, VEGF. In some embodiments, the at least one immunostimulatory agent comprises an antagonist of a chemokine such as CXCR2, CXCR4, CCR2 or CCR4. In some embodiments, the immunostimulatory agent is or comprises an agonist of a cytokine that stimulates T cell activation, such as IL-2, IL-7, IL-12, IL-15, IL-21 and IFNα.

In preferred embodiments, the immunostimulatory agent is selected from the group consisting of a TLR7 agonist, a TLR8 agonist, a TLR7 antagonist, a TLR8 antagonist, a Sting inhibitor, a TGF beta inhibitor, an a2A inhibitor and an a2B inhibitor.

Alternatively, the therapeutic agent may be an immunosuppressive agent.

As used herein, an "immunosuppressive agent" is an agent that inhibits the development or maintenance of an immunologic response. Such inhibition by an immunosuppressive agent can be effected by, for example, elimination of immune cells (e.g., T or B lymphocytes); induction or generation of immune cells that can modulate (e.g., down-regulate) the functional capacity of other cells; induction of an unresponsive state in immune cells (e.g., anergy); or increasing, decreasing or changing the activity or function of immune cells, including, for example, altering the pattern of proteins expressed by these cells (e.g., altered production and/or secretion of certain classes of molecules such as cytokines, chemokines, growth factors, transcription factors, kinases, costimulatory molecules or other cell surface receptors, and the like). In typical embodiments, an immunosuppressive agent has a cytotoxic or cytostatic effect on an immune cell that promotes an immune response. In some embodiments, said immunosuppressive agent results in the reduction of an undesired immune response as compared to the administration of a control molecule that does not include said immunosuppressive agent.

"Immune cell", as used herein, means any cell of hematopoietic lineage involved in regulating an immune response against an antigen (e.g., an autoantigen), such as a T cell (T lymphocyte), a B cell (B lymphocyte) or a dendritic cell. Preferably, an immune cell according to the present disclosure is a T cell or B cell.

In preferred embodiments, the immunosuppressive agent according to the present disclosure is selected from the group consisting of an IMDH (inosine monophosphate dehydrogenase) inhibitor, an mTor (mechanistic target of rapamycin) inhibitor, a SYK (spleen tyrosine kinase) inhibitor, a JAK (janus kinase) inhibitor and a calcineurin inhibitor.

As a further alternative, the Functional agent can be a detectable label.

As used herein, "detectable label" refers to a molecule capable of detection (i.e. capable of being detected by methods known in the art, e.g. detection methods based on radiography, fluorescence, chemiluminescence, enzymatic activity or absorbance).

A compound according to the present disclosure with a detectable label as Functional agent (e.g. an ADC according to the present disclosure with a detectable label as payload), can be useful for diagnosing a disease, identifying the site of a disease, or for monitoring or prognosing the onset, development, progression and/or severity of a disease or disorder as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. There are even situations where a detectable label and a therapeutic agent can be used in combination (e.g. Rondon and Degoul, 2020).

A detectable label as Functional agent may e.g. be an enzyme (such as horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase), a prosthetic group (such as streptavidin/biotin and avidin/biotin), a fluorescent material (such as Alexa Fluor® 350, Alexa Fluor® 405, Alexa Fluor® 430, Alexa Fluor® 488, Alexa Fluor® 500, Alexa Fluor® 514, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 610, Alexa Fluor® 633, Alexa Fluor® 647, Alexa Fluor® 660, Alexa Fluor® 680, Alexa Fluor® 700, Alexa Fluor® 750, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin), a luminescent material (such as luminol), a bioluminescent material (such as luciferase, luciferin or aequorin), a radioactive material (such as iodine (^{131}I, ^{125}I, ^{123}I or ^{121}I),

carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115}In, ^{113}In, ^{112}In or ^{111}In), technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{64}Cu, ^{113}Sn, and ^{117}Sn), a positron-emitting metal (for positron emission tomography), or a non-radioactive paramagnetic metal ion. Alternatively, a detectable label can for example be a fluorophore, a spin label, an infrared probe, an affinity probe, a spectroscopic probe, a radioactive probe, or a quantum dot.

Preferably, said detectable label is a radioisotope, fluorophore, chromophore, enzyme, dye, metal ion, ligand (such as biotin, avidin, streptavidin or hapten) or quantum dot.

Within a Linker-Functional agent unit, there may be one Functional agent or more than one Functional agent (as defined by the parameter *f*, wherein $f > 1$ indicates the presence of several Functional agents). Moreover, there may be more than one Linker-Functional agent unit per compound (as defined by the parameter *p*). At each such occurrence of the Functional agent F, the structure of the Functional agent is independently selected within the structural limits defined herein.

Preferably, all Functional agents present in a compound according to the present disclosure have the same chemical structure.

Preparation of compound according to the present disclosure

The compound of the present disclosure can be prepared by standard methods of synthetic organic chemistry known to the skilled person and standard methods of genetic engineering and recombinant protein technology known to the skilled person (see e.g. Green and Sambrook, "Molecular Cloning: A Laboratory Manual", 2014; Coligan et al., "Current Protocols in Protein Science", 1997). Exemplary methods are also described in the Examples section of the present disclosure.

General methods for the preparation of compounds like ADCs by different approaches can be found for example in Matsuda et al., Organic Process Research & Development (2019), vol. 23(12), p. 2647-2654; Walker et al., Bioconjugate Chemistry (2019), vol. 30(9), p. 2452-2457; Barfield and Rabuka, Methods in Molecular Biology (2018), vol. 1728 (Noncanonical Amino Acids), p. 3-16.

Individual parts of the compound can be prepared separately and later covalently coupled by methods for covalently conjugating chemical compounds that are known to a person of skill in the art of chemical synthesis. For example, if the compound according to the present disclosure is an ADC, a drug-linker construct can be prepared by chemical synthesis and this drug-linker construct can then subsequently be conjugated to an antibody as Ligand unit by a chemical reaction with appropriate reactive groups (e.g. linkage by maleimide chemistry) or by enzymatic linkage (e.g. transglutaminase-catalyzed linkage).

By use of orthogonal conjugation methods for the successive linkage of different components (such as combination of transglutaminase and cysteine conjugation), a high level of control can be achieved.

If one of the components to be covalently linked contains or is a protein, care must be taken not to damage the protein during the conjugation. While the reaction environment during enzymatic addition is typically not harmful to a protein component like an antibody, the conditions for attachment by chemical reaction must be appropriately chosen to avoid damage to the protein components. Conjugation to a sensitive component may for example be carried out via a cycloaddition reaction such as Click Chemistry or Diels Alder type modifications (Rossin et al., Bioconjugate Chemistry (2016), vol. 27(7), p. 1697-1706) or using aldehydes (Barfield and Rabuka, Methods in Molecular Biology (2018), vol. 1728, p. 3-16). Suitable conditions can also be inferred from the conditions for chemical linker conjugation to the antibody component as described in Example 1 below.

Chemical linkage of two previously prepared individual molecular parts of the compound according to the present disclosure (a first and a second molecule) may be carried out by use of an activated intermediate compound. Such an activated intermediate compound may be obtained by introducing an activator group to the first or second molecule by standard methods of organic synthesis.

The activator group is a reactive functional group. The activator group may for example be an activator group for chemical coupling such as a maleimide, an *N*-hydroxysuccinimide ester, a halogen-acetamide, an alkyl halogen, a Michael acceptor (wherein said Michael acceptor is preferably a vinyl-pyridine) and a group suitable for cycloaddition (wherein said group suitable

for cycloaddition is preferably a ketone, hydrazone, semicarbazone, carboxylic acid, alkene or alkyne suitable for cycloaddition). Alternatively, the activator group may be an activator group for enzymatic coupling, such as a triple glycine GlyGlyGly (for sortase oder transglutaminase coupling) or a primary amine (for transglutaminase coupling). Subsequently, the activated intermediate compound is covalently linked to the second molecular part of the compound by a reaction of said activator group with an appropriate molecular group within the second part of the compound.

Successful covalent linkage of the components can then be confirmed by standard methods known to a skilled person, e.g. by LC-MS.

Alternatively, intermediates of the ADC synthesis (e.g. a construct comprising a linker and payload) may be built not by covalently linking previously prepared components, but by gradually synthesizing the complete construct in one molecule. The synthetic sequence and its flexibility is driven be the desired structure and various approaches have been described (e.g. Quiles et al., Journal of Medicinal Chemistry (2010), vol. 53(2), p. 586-594; Feuillatre et al., ACS Omega (2020), vol. 5(3), p. 1557-1565; Sonzini, Bioconjugate Chemistry (2020), vol. 31(1), p. 123-129; Watkinson, BioProcess International (2017), vol. 15(10), p. 22-33).

Exemplary methods for the preparation of a compound according to the present disclosure are also described in detail in the Examples section of the present disclosure.

Embodiment 9: The compound according to any one of embodiments 1 to 8, wherein said Iduronide unit, said Stretcher unit (if present) and said Spacer unit (if present) form a Linker unit.

Embodiment 10: The compound according to any one of embodiments 5 to 9, wherein said Linker unit covalently links the Ligand unit and the Functional agent.

Embodiment 11: The compound according to any one of embodiments 5 to 10, wherein the presence of said Linker unit in said compound does not interfere with the specific binding properties of the Ligand unit.

Embodiment 12: The compound according to any one of embodiments 1 to 11, wherein said Linker unit is cleavable by enzymatic cleavage with iduronidase.

Embodiment 13: The compound according to any one of embodiments 1 to 12, wherein said Linker unit remains uncleaved until the compound has been recruited to its target site.

Embodiment 14: The compound according to any one of embodiments 3 to 13, wherein the presence of said Linker unit in said compound does not interfere with the function of the Functional agent.

Embodiment 15: The compound according to any one of embodiments 1 to 14, wherein said compound is cleavable by enzymatic cleavage.

Embodiment 16: The compound according to any one of embodiments 1 to 15, wherein said Linker unit is cleavable by enzymatic cleavage.

As used herein, a Linker unit or compound that is "cleavable by enzymatic cleavage" is a Linker unit/compound that is cleaved in the presence of a certain enzyme, but stable in the absence of this enzyme. For the purposes of the compound of the present disclosure, this enzyme will typically be iduronidase, preferably human α-L-iduronidase. The compound is not exposed to iduronidase in the extracellular environment (because iduronidase is not present there), but exposed to iduronidase upon uptake of the compound into the target cells (in which iduronidase is present), resulting in a Linker unit that is extracellularly stable, but cleaved upon entry into the target cell.

Embodiment 17: The compound according to any one of embodiments 1 to 16, wherein said Linker unit can be cleaved by enzymatic cleavage with iduronidase enzyme.

Embodiment 18: The compound according to any one of embodiments 1 to 17, wherein said Linker unit is not cleavable by a glucuronidase.

Embodiment 19: The compound according to any one of embodiments 1 to 18, wherein said Linker unit is not cleavable by enzymatic cleavage with human glucuronidase. A glucuronidase is an enzyme of E.C. 3.2.1.31 that catalzyes the cleavage of β-Glucuronides.

Embodiment 20: The compound according to any one of embodiments 1 to 19, wherein said Linker unit is not cleavable by any sugar hydrolase other than iduronidase.

Embodiment 21: The compound according to any one of embodiments 1 to 20, wherein an iduronidase is an enzyme that catalyzes the hydrolysis of iduronosidic linkages.

Embodiment 22: The compound according to any one of embodiments 1 to 21, wherein, once the compound comes into contact with iduronidase, the connection that the Linker unit provides between the Ligand unit and the Functional agent is disrupted (and, preferably, the Functional agent is released).

Embodiment 23: The compound according to any one of embodiments 1 to 22, wherein said Linker unit is not cleavable by enzymatic cleavage with any human glycosidase other than human iduronidase.

Embodiment 24: The compound according to any one of embodiments 1 to 23, wherein said Linker unit is cleaved upon exposure to the intracellular environment of a cell that expresses human α-L-iduronidase.

Embodiment 25: The compound according to any one of embodiments 1 to 24, wherein a Linker unit that is "cleaved upon exposure to the intracellular environment of a cell that expresses human α-L-iduronidase" is a Linker unit that has such a structure that if compounds including this Linker unit enter into the intracellular environment of cells that express human α-L-iduronidase, after an incubation of 24 h at 37 °C at least 60%, preferably at least 75% of the Linker units in the compounds have been cleaved.

Embodiment 26: The compound according to any one of embodiments 1 to 25, wherein cleavage of the Iduronide unit with iduronidase causes breaking of the covalent linkage between the Functional agent and the Ligand unit that is provided by the Linker unit (and, preferably, release of the Functional agent into the intracellular environment).

Whether the Linker unit of a compound is stable or cleaved may be examined by exposing the compound to the conditions to be tested (e.g. human serum) for a defined period of time and

then verifying the integrity of the linker in the treated sample and an untreated control sample by standard analytical techniques such as mass spectroscopy, HPLC, and the separation/analysis technique LC/MS.

More specifically, whether a Linker unit is cleaved upon exposure to the intracellular environment of cells that express human α-L-iduronidase can e.g. be determined as described in Example 9. Alternatively, a cleavage assay as described above can be carried out with cell lysates of cells that express human α-L-iduronidase vs. with cell lysates of cells that do not express human α-L-iduronidase.

For many cell types, it is known from the literature if they express human α-L-iduronidase. Otherwise, whether a cell type expresses human α-L-iduronidase can be verified by Western blotting with total cell extracts of cells of this cell type using an antibody that is specific for human α-L-iduronidase.

Embodiment 27: The compound according to any one of embodiments 1 to 26, wherein said Linker unit is stable in the absence of human α-L-iduronidase.

Embodiment 28: The compound according to any one of embodiments 1 to 27, wherein said Linker unit is stable in the extracellular space of the human body.

Embodiment 29: The compound according to embodiment 28, wherein a Linker unit is "stable in the extracellular space of the human body" is a Linker unit that has such a structure that after an incubation of 24 h at 37 °C in human serum, at least 60%, preferably at least 75% of the Linker units in the compounds has been neither cleaved nor degraded.

Whether a Linker unit is stable in the extracellular space of the human body can be determined, for example, by incubating independently with serum both (a) the compound (the "Compound sample") and (b) an equal molar amount of unconjugated Ligand or unconjugated Ligand and Functional agent (the "Control sample") for a predetermined time period (e.g. 24 hours) and then comparing the amount of unconjugated Ligand or Functional agent present in the Compound sample with that present in the Control sample, as measured, for example, by high performance liquid chromatography. See also Example 9 below.

Embodiment 30: The compound according to any one of embodiments 1 to 29, wherein said Linker unit is stable in the intracellular environment of cells that do not express human α-L-iduronidase.

Embodiment 31: The compound according to embodiment 30, wherein a Linker unit that is "stable in the intracellular environment of cells that do not express human α-L-iduronidase" is a Linker unit that has such a structure that if compounds including this Linker unit enter into the intracellular environment of cells that do not express human α-L-iduronidase, after an incubation of 24 h at 37 °C at least 60%, preferably at least 75% of the Linker units in the compounds have been neither cleaved nor degraded.

Whether a Linker unit is "stable in the intracellular environment of cells that do not express human α-L-iduronidase" can e.g. be determined as described in Example 8. Alternatively, a cleavage assay as described above can be carried out with cell lysates of cells that do not express human α-L-iduronidase vs. with cell lysates of cells that do express human α-L-iduronidase.

Embodiment 32: The compound according to any one of embodiments 1 to 31, wherein said Linker unit is stable in the extracellular space of the human body and stable in the intracellular environment of cells that do not express human α-L-iduronidase, but cleavable cleaved upon exposure to the intracellular environment of a cell.

Embodiment 33: The compound according to any one of embodiments 1 to 32, wherein said cleavage of the Linker unit releases the Functional agent from the compound into the intracellular environment of cells that express human α-L-iduronidase.

Embodiment 34: The compound according to any one of embodiments 5 to 33, wherein the Ligand unit specifically binds to a cell that expresses iduronidase enzyme in its cytoplasm.

Embodiment 35: The compound according to embodiment 34, wherein said cell is a cell in which the enzyme human α-L-iduronidase is present.

Embodiment 36: The compound according to any one of embodiments 34 to 35, wherein said cell is a cell that expresses the enzyme human α-L-iduronidase.

Embodiment 37: The compound according to any one of embodiments 34 to 36, wherein in the intracellular environment of said cell the enzyme human α-L-iduronidase is present.

Embodiment 38: The compound according to any one of embodiments 1 to 37, wherein said iduronidase is human α-L-iduronidase.

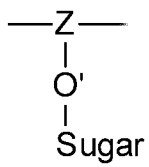
Embodiment 39: The compound according to any one of embodiments 1 to 38, wherein said iduronidase is α-L-iduronidase.

Embodiment 40: The compound according to any one of embodiments 1 to 39, wherein said Iduronide unit

- links a Stretcher unit to a Spacer unit if Stretcher and Spacer units are present in said compound,
- links a Stretcher unit to the Functional agent, if a Stretcher unit is present and no Spacer unit is present in said compound,
- links the Ligand unit to a Spacer unit, if no Stretcher unit is present but a Spacer unit is present in said compound,
- links the Ligand unit to the Functional agent if neither a Stretcher unit nor a Spacer unit is present in said compound.

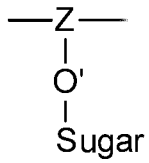
Embodiment 41: The compound according to any one of embodiments 1 to 40, wherein said Iduronide unit is a chemical group that comprises an iduronide.

Embodiment 42: The compound according to any one of embodiments 1 to 41, wherein said Iduronide unit (W) comprises a sugar moiety ("Sugar") linked via a glycosidic bond (–O'–) to a self-immolative group (Z), according to the formula:



(Formula 9).

Embodiment 43: The compound according to any one of embodiments 1 to 42, wherein said Iduronide unit is a sugar moiety ("Sugar") linked via a glycosidic bond (–O'–) to a self-immolative group (Z), according to the formula:



(Formula 9).

Embodiment 44: The compound according to any one of embodiments 1 to 43, wherein said self-immolative group is a chemical moiety that provides a covalent link between said Functional agent and said Ligand unit (either directly or indirectly via Spacer unit(s) and/or Stretcher unit(s)).

Embodiment 45: The compound according to any one of embodiments 1 to 44, wherein, upon cleavage of the glycosidic bond that links said self-immolative group to said sugar moiety, said self-immolative group decomposes in such a way that the covalent link between said Functional agent and said Ligand unit is disrupted.

Embodiment 46: The compound according to any one of embodiments 1 to 45, wherein, upon cleavage of the glycosidic bond that links said self-immolative group to said sugar moiety, said self-immolative group decomposes in such a way that the covalent link between said Functional agent and said Ligand unit is disrupted and the Functional agent is released.

Embodiment 47: The compound according to any one of embodiments 1 to 46, wherein, once the glycosidic bond between the self-immolative group and the sugar moiety is cleaved by enzymatic cleavage with iduronidase, the self-immolative group degrades in such a manner that also the linkage between the Functional agent and the Ligand unit is disrupted and the Functional agent is released.

Embodiment 48: The compound according to any one of embodiments 1 to 47, wherein said Iduronide unit comprises an iduronidase cleavage site. An iduronidase cleavage site is a site that can be cleaved enzymatically by iduronidase.

Embodiment 49: The compound according to any one of embodiments 1 to 48, wherein said Iduronide unit is cleavable by iduronidase.

Embodiment 50: The compound according to any one of embodiments 1 to 49, wherein said self-immolative group is a di-functional chemical moiety that is capable of covalently linking the sugar moiety and the Functional agent.

Embodiment 51: The compound according to any one of embodiments 1 to 50, wherein said self-immolative group is a di-functional chemical moiety that is capable of covalently linking the sugar moiety and the Functional agent, wherein said self-immolative group binds to the sugar moiety via a glycosidic bond and to the Functional agent either directly without a Spacer unit or indirectly via a Spacer unit.

Embodiment 52: The compound according to any one of embodiments 1 to 51, wherein said self-immolative group is a tri-functional chemical moiety that is capable of covalently linking the sugar moiety, the Functional agent and the Ligand unit.

Embodiment 53: The compound according to any one of embodiments 1 to 52, wherein said self-immolative group is a tri-functional chemical moiety that is capable of covalently linking the sugar moiety (via a glycosidic bond), the Functional agent (directly without a Space unit or indirectly via a Spacer unit) and the Ligand unit (directly without a Stretcher unit or indirectly via a Stretcher unit).

Embodiment 54: The compound according to any one of embodiments 1 to 53, wherein said self-immolative group spontaneously separates from the Functional agent (in the absence of a Spacer unit) or the Spacer unit (in the presence of a Spacer unit) if the bond of the self-immolative group to the sugar moiety is cleaved.

Embodiment 55: The compound according to any one of embodiments 1 to 54, wherein said self-immolative group spontaneously decomposes if the Iduronide unit is cleaved by iduronidase, preferably, resulting in breaking of the covalent linkage between the Ligand unit and the Functional agent.

Embodiment 56: The compound according to any one of embodiments 6 to 55, wherein said sugar moiety is cyclic hexose, preferably a pyranose.

Embodiment 57: The compound according to any one of embodiments 6 to 56, wherein the sugar moiety is an iduronide moiety (i.e. an α-L-iduronic acid or β-L-iduronic acid linked to the self-immolative group Z via a glycosidic bond that is cleavable by iduronidase).

Embodiment 58: The compound according to any one of embodiments 6 to 57, wherein the sugar moiety is an α-L-iduronic acid or β-L-iduronic acid linked to the self-immolative group Z via a glycosidic bond.

Embodiment 59: The compound according to any one of embodiments 6 to 58, wherein the glycosidic bond (–O'–) is an iduronidase cleavage site.

Embodiment 60: The compound according to any one of embodiments 6 to 59, wherein the Iduronide unit undergoes self-immolation upon cleavage at the iduronidase cleavage site.

More information about self-immolation and chemical groups suitable for this purpose can be found e.g. in WO 03/026577 A2 or WO 2021/207701 A1.

As used herein, if the Iduronide unit "undergoes self-immolation" (or if a chemical group is a "self-immolative" chemical group), this preferably means that said Iduronide unit/chemical group undergoes, upon cleavage of the chemical bond that triggers self-immolation, intramolecular cleavage with a half-life of below 12 hours (preferably below 4 hours, more preferably below 2 hours) in water at 37° C at a pH of 7.4.

Embodiment 61: The compound according to any one of embodiments 6 to 60, wherein the glycosidic bond (–O'–) is a bond cleavable by human α-L-iduronidase.

Embodiment 62: The compound according to any one of embodiments 6 to 61, wherein the sugar moiety is an α-L-iduronide moiety (i.e. an α-L-iduronic acid linked to the self-immolative group Z via a glycosidic bond that is cleavable by α-L-iduronidase).

Embodiment 63: The compound according to any one of embodiments 6 to 62, wherein the sugar moiety is an α-L-iduronic acid linked to the self-immolative group Z via a glycosidic bond.

Embodiment 64: The compound according to any one of embodiments 6 to 63, wherein the sugar moiety is an α-L-iduronide or β-L-iduronide.

Embodiment 65: The compound according to any one of embodiments 6 to 64, wherein the sugar moiety is an α-L-iduronide.

Embodiment 66: The compound according to any one of embodiments 6 to 65, wherein the sugar moiety is an α-L-iduronic acid.

Embodiment 67: The compound according to any one of embodiments 6 to 66, wherein the sugar moiety is linked to the self-immolative group Z.

Embodiment 68: The compound according to any one of embodiments 6 to 67, wherein the sugar moiety is linked to the self-immolative group Z via a glycosidic bond.

Embodiment 69: The compound according to any one of embodiments 6 to 68, wherein said glycosidic bond is cleavable by iduronidase.

Embodiment 70: The compound according to any one of embodiments 6 to 69, wherein said glycosidic bond is cleavable by α-L-iduronidase.

Embodiment 71: The compound according to any one of embodiments 6 to 70, wherein said glycosidic bond is cleavable by human α-L-iduronidase.

Embodiment 72: The compound according to any one of embodiments 1 to 71, wherein said Linker unit is cleavable by enzymatic cleavage with human α-L-iduronidase.

Embodiment 73: The compound according to any one of embodiments 1 to 72, wherein said Iduronide unit includes a site that can be cleaved enzymatically by an iduronidase enzyme.

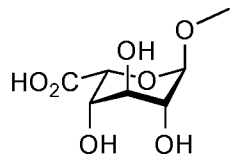
Embodiment 74: The compound according to any one of embodiments 1 to 73, wherein said compound comprises only one type of Iduronide unit. This means that all Iduronide units that are part of the compound are identical (they are of the same kind with regard to their molecular structure).

Embodiment 75: The compound according to any one of embodiments 1 to 73, wherein said compound comprises more than one kind of Iduronide unit. This means that there are at least two different types of Iduronide unit with different structure covalently attached (either directly or indirectly) to the Ligand unit (i.e. the Iduronide units covalently attached to the Ligand unit are of more than one kind with regard to their molecular structure).

Embodiment 76: The compound according to any one of embodiments 1 to 73 or 75, wherein said compound comprises up to two kinds of Iduronide units.

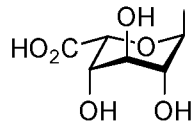
Sugar moiety

Embodiment 77: The compound according to any one of embodiments 6 to 76, wherein the sugar moiety (with glycosidic bond) has the following structure:



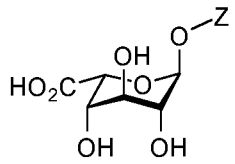
(Formula 17).

Embodiment 78: The compound according to any one of embodiments 6 to 76, wherein the sugar moiety has the following structure:



(Formula 18).

Embodiment 79: The compound according to any one of embodiments 6 to 76, wherein the Iduronide unit has the following structure:



(Formula 19).

Embodiment 80: The compound according to any one of embodiments 6 to 79, wherein the sugar moiety is unsubstituted (e.g., a naturally occurring cyclic hexose).

Embodiment 81: The compound according to any one of embodiments 6 to 79, wherein the sugar moiety is a substituted α-L-iduronide.

Embodiment 82: The compound according to embodiment 81, wherein the substituted α-L-iduronide is iduronic acid substituted with one or more chemical groups.

Embodiment 83: The compound according to embodiment 82, wherein said one or more chemical groups are selected from the group consisting of hydrogen, hydroxyl, halogen, sulfur, nitrogen and lower alkyl.

Embodiment 84: The compound according to embodiment 83, wherein said lower alkyl is C_1 to C_5, preferably C_1 to C_3.

Embodiment 85: The compound according to any one of embodiments 6 to 84, wherein the self-immolative group Z is a meta-aminobenzyl alcohol unit.

Embodiment 86: The compound according to any one of embodiments 6 to 85, wherein the self-immolative group Z is a para-aminobenzyl alcohol unit.

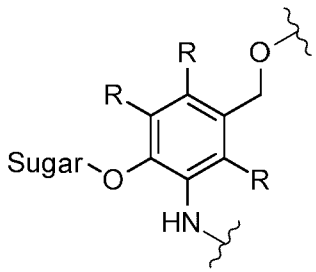
Embodiment 87: The compound according to any one of embodiments 6 to 86, wherein the self-immolative group (Z) is covalently linked to the sugar moiety, to the Functional agent, and to the Ligand.

Embodiment 88: The compound according to any one of embodiments 6 to 87, wherein the self-immolative group (Z) is covalently linked to the sugar moiety, to the Functional agent

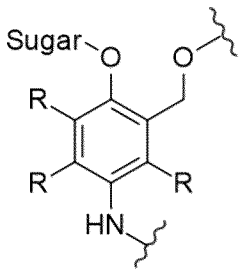
(directly or indirectly via the Spacer unit(s)), and to the Ligand (directly or indirectly via the Stretcher unit(s)).

Embodiment 89: The compound according to any one of embodiments 5 to 88, wherein said Ligand unit comprises a targeting ligand.

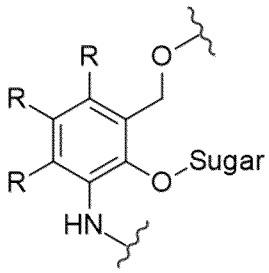
Embodiment 90: The compound according to any one of embodiments 1 to 89, wherein the Iduronide unit has a structure according to a formula selected from the group consisting of the following formulas:



(Formula 20)



(Formula 21)



(Formula 22),

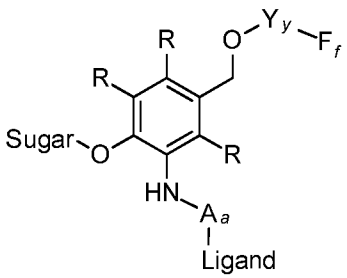
wherein

Sugar is an iduronide; and

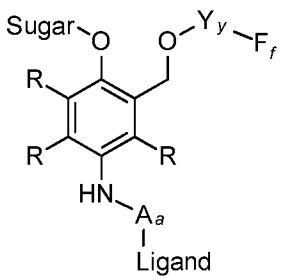
each R is independently selected from the group consisting of hydrogen, halo (preferably chloro, bromo or fluoro), —CN, —NO_2, and another electron-

withdrawing or electron-donating group, provided that the Iduronide unit undergoes self-immolation upon cleavage of the glycosidic bond.

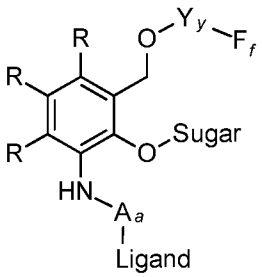
Embodiment 91: The compound according to any one of embodiments 1 to 90, wherein said compound comprises a structure according to a formula selected from the group consisting of the following formulas:



(Formula 23)



(Formula 24)



(Formula 25),

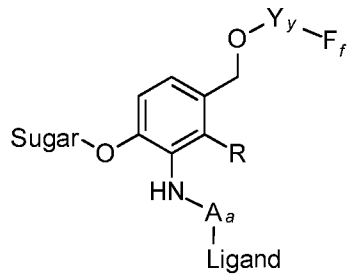
wherein

Sugar is an iduronide; and

each R is independently selected from the group consisting of hydrogen, halo (preferably chloro, bromo or fluoro), —CN, —NO_2, and another electron-

withdrawing or electron-donating group, provided that the Iduronide unit undergoes self-immolation upon cleavage of the glycosidic bond.

Embodiment 92: The compound according to any one of embodiments 1 to 91, wherein said compound comprises a structure according to the following formula:



(Formula 26).

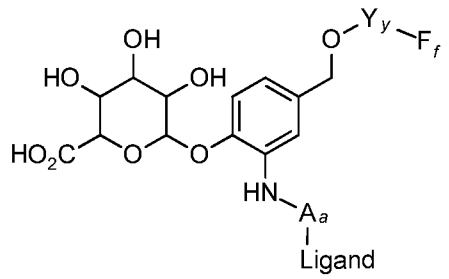
Embodiment 93: The compound according to any one of embodiments 1 to 92, wherein the glycosidic bond (—O'—) comprises the oxygen bond between the Sugar and the self-immolative group Z.

Embodiment 94: The compound according to any one of embodiments 1 to 93, wherein each R is independently selected from the group consisting of hydrogen, halo (preferably chloro or bromo, fluoro,), —CN and —NO_2.

Embodiment 95: The compound according to any one of embodiments 1 to 93, wherein each R is independently selected from the group consisting of hydrogen, chloro, bromo, fluoro, —CN and —NO_2.

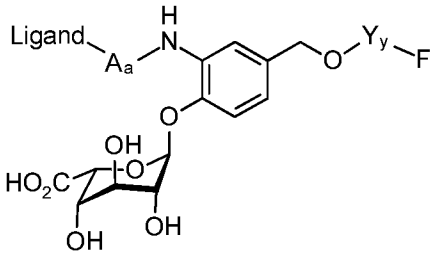
Embodiment 96: The compound according to any one of embodiments 1 to 93, wherein each R is hydrogen.

Embodiment 97: The compound according to any one of embodiments 1 to 96, wherein said compound comprises a structure according to the following formula:



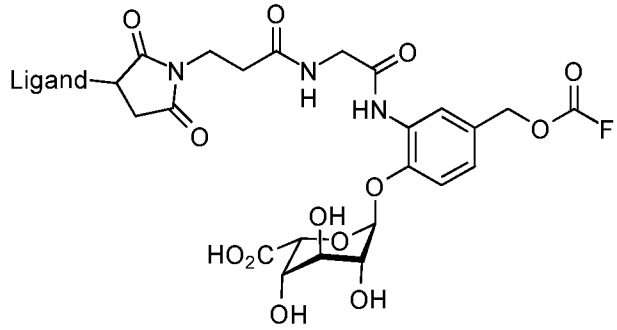
(Formula 27).

Embodiment 98: The compound according to any one of embodiments 1 to 97, wherein said compound comprises a structure according to the following formula:



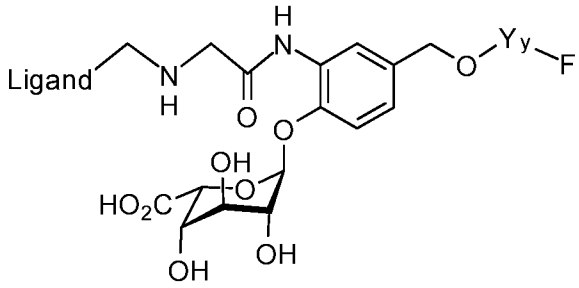
(Formula 28).

Embodiment 99: The compound according to any one of embodiments 1 to 98, wherein said compound comprises a structure according to the following formula:



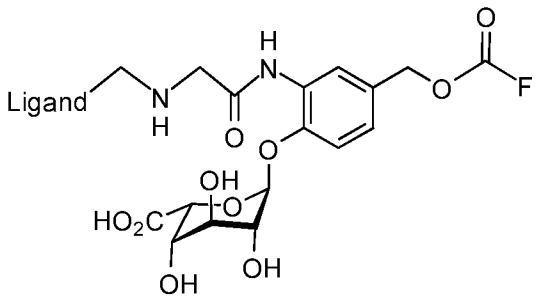
(Formula 29).

Embodiment 100: The compound according to any one of embodiments 1 to 99, wherein said compound comprises a structure according to the following formula:



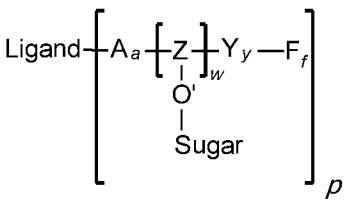
(Formula 30).

Embodiment 101: The compound according to any one of embodiments 1 to 100, wherein said compound comprises a structure according to the following formula:



(Formula 31).

Embodiment 102: The compound according to any one of embodiments 1 to 101, wherein the Ligand unit is linked (directly or indirectly) to the sugar moiety, which is linked to the self-immolative group (Z) which is linked (directly or indirectly) to the Functional agent, according to the following formula:

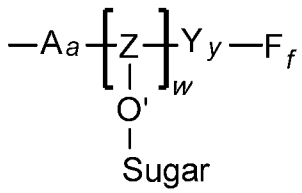


(Formula 32).

Embodiment 103: The compound according to any one of embodiments 1 to 102, wherein the sugar moiety is linked directly to the Ligand unit or wherein the sugar moiety is linked to the Ligand unit indirectly via a Stretcher unit.

Embodiment 104: The compound according to any one of embodiments 1 to 103, wherein the self-immolative group (Z) is linked directly to the Functional agent or wherein the self-immolative group (Z) is linked to the Functional agent (F) indirectly via a Spacer unit.

Embodiment 105: The compound according to any one of embodiments 1 to 104, wherein said Linker unit and said Functional agent form a Linker-Functional agent unit with the following formula:



(Formula 7).

Embodiment 106: The compound according to any one of embodiments 1 to 105, wherein said compound comprises at least one Linker-Functional agent unit.

Embodiment 107: The compound according to any one of embodiments 1 to 106, wherein said compound comprises at least 2 Linker-Functional agent units.

Embodiment 108: The compound according to any one of embodiments 1 to 107, wherein said compound comprises at least 4 Linker-Functional agent units.

Embodiment 109: The compound according to any one of embodiments 1 to 108, wherein said compound comprises at least 6 Linker-Functional agent units.

Embodiment 110: The compound according to any one of embodiments 1 to 109, wherein said compound comprises at least 8 Linker-Functional agent units.

Embodiment 111: The compound according to any one of embodiments 1 to 110, wherein said compound comprises up to 20 Linker-Functional agent units.

Embodiment 112: The compound according to any one of embodiments 1 to 111, wherein said compound comprises up to 10 Linker-Functional agent units.

Embodiment 113: The compound according to any one of embodiments 1 to 112, wherein said compound comprises up to 8 Linker-Functional agent units.

Embodiment 114: The compound according to any one of embodiments 1 to 113, wherein said compound comprises up to 6 Linker-Functional agent units.

Embodiment 115: The compound according to any one of embodiments 1 to 114, wherein said compound comprises up to 4 Linker-Functional agent units.

Embodiment 116: The compound according to any one of embodiments 1 to 115, wherein said compound comprises up to 2 Linker-Functional agent units.

Embodiment 117: The compound according to any one of embodiments 5 to 116, wherein said Ligand unit is a moiety (i.e. a molecular group or chemical structure) that is capable of directing said compound to a target site.

Embodiment 118: The compound according to embodiment 117, wherein said target site is a binding site on a target cell.

Embodiment 119: The compound according to any one of embodiments 117 to 118, wherein said target site is a biological molecule or a part of a biological molecule.

Embodiment 120: The compound according to any one of embodiments 118 to 119, wherein by binding to said biological molecule or part thereof, the Ligand unit directs the compound comprising said Ligand unit to said binding site.

Embodiment 121: The compound according to any one of embodiments 117 to 120, wherein due to the interaction of the Ligand unit with its target site, said compound is concentrated preferentially at its site target site.

Embodiment 122: The compound according to any one of embodiments 5 to 121, wherein said Ligand unit is covalently linked to the Linker unit.

Embodiment 123: The compound according to any one of embodiments 5 to 122, wherein said Ligand unit is covalently linked to the Iduronide unit, either directly or indirectly via a Stretcher unit, if present.

Embodiment 124: The compound according to any one of embodiments 5 to 123, wherein said Ligand unit specifically binds to a desired binding site in such a manner that it can recruit the compound to a target site.

Embodiment 125: The compound according to any one of embodiments 5 to 124, wherein said Ligand unit comprises a protein, a peptide, a peptide mimetic, a nucleic acid, an oligonucleotide or a small molecule.

Embodiment 126: The compound according to any one of embodiments 5 to 125, wherein said Ligand unit is selected from the group consisting of a protein, a peptide, a peptide mimetic, a nucleic acid, an oligonucleotide and a small molecule.

Embodiment 127: The compound according to any one of embodiments 5 to 125, wherein said Ligand unit comprises a protein.

By stating that the Ligand unit "comprises" a protein, the present disclosure designates that the Ligand unit includes a part within its chemical structure that is a protein. A Ligand unit that comprises a protein may or may not comprise a part that is not a protein.

Embodiment 128: The compound according to any one of embodiments 5 to 127, wherein said Ligand unit is a protein.

By stating that the Ligand unit "is" a protein, the present disclosure designates that the Ligand unit consists only of protein and does not comprise a part that is not a protein.

Embodiment 129: The compound according to any one of embodiments 5 to 128, wherein said Ligand unit comprises or is a protein which is a protein ligand that specifically binds to a receptor at the surface of a cell.

Embodiment 130: The compound according to any one of embodiments 5 to 129, wherein said Ligand unit comprises or is a protein which is an antibody or an antigen-binding fragment thereof.

Embodiment 131: The compound according to any one of embodiments 5 to 130, wherein said Ligand unit comprises or is a protein which is an antibody.

Embodiment 132: The compound according to any one of embodiments 5 to 127 or 129 to 131, wherein said Ligand unit comprises or is a small molecule.

Embodiment 133: The compound according to any one of embodiments 5 to 127 or 129 to 132, wherein said Ligand unit comprises a small molecule.

Embodiment 134: The compound according to any one of embodiments 5 to 126 or 130 to 133, wherein said Ligand unit is a small molecule.

Embodiment 135: The compound according to any one of embodiments 1 to 134, wherein a small molecule is a molecule with a molecular weight < 1000 Da.

Embodiment 136: The compound according to any one of embodiments 5 to 133 or 135, wherein said Ligand unit (L) comprises an antibody.

Embodiment 137: The compound according to any one of embodiments 5 to 133 or 135 to 136, wherein said Ligand unit (L) is an antibody.

Embodiment 138: The compound according to any one of embodiments 1 to 137, wherein said antibody is selected from the group consisting of an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, IgG4 antibody, an IgA antibody, an IgM antibody, and hybrids thereof.

Embodiment 139: The compound according to any one of embodiments 1 to 137, wherein said antibody is selected from the group consisting of an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, an IgG4 antibody, and hybrids thereof.

Embodiment 140: The compound according to any one of embodiments 1 to 137, wherein said antibody is selected from the group consisting of an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, and an IgG4 antibody.

Embodiment 141: The compound according to any one of embodiments 1 to 137, wherein said antibody is an IgG1 antibody.

Embodiment 142: The compound according to any one of embodiments 1 to 141, wherein said antibody is a monoclonal antibody or a polyclonal antibody.

Embodiment 143: The compound according to any one of embodiments 1 to 141, wherein said antibody is a monoclonal antibody.

Embodiment 144: The compound according to any one of embodiments 1 to 143, wherein said antibody is a full-length antibody or an antigen-binding fragment thereof.

Embodiment 145: The compound according to any one of embodiments 1 to 143, wherein said antibody is a full-length antibody.

Embodiment 146: The compound according to any one of embodiments 1 to 143, wherein said antibody is an antigen-binding fragment of a full-length antibody.

Embodiment 147: The compound according to any one of embodiments 130 to 146, wherein said antigen-binding fragment is selected from the group consisting of a Fab, a Fab', a (Fab')2 and a Fv.

Embodiment 148: The compound according to any one of embodiments 130 to 147, wherein said antigen-binding fragment is selected from the group consisting of a scFv, a diabody and a VHH.

Embodiment 149: The compound according to any one of embodiments 5 to 148, wherein said Ligand unit/said antibody (resp. said antibody that is included as Ligand unit in said compound) is capable of specifically binding to an antigen that is present on the surface of a target cell.

As used herein, an "antigen that is present on the surface of a target cell" is an antigen that is present on the surface of the target cell in such a manner that it is accessible from the extracellular environment (i.e. an antibody can bind to it from the extracellular environment). For example, CD8 is a transmembrane protein of cytotoxic T cells, and its extracellular domain is accessible for antibodies directed against the extracellular domain of CD8 from the extracellular environment. Thus, in the sense of the present disclosure, CD8 is an antigen that is present on the surface of cytotoxic T cells. In an embodiment, said "antigen that is present on the surface of a target cell" is a protein that is present on the surface of a target cell.

An antibody "binds" an antigen of interest is an antibody that is capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting to a cell expressing the antigen.

If the present disclosure refers to a first molecule/molecular group (e.g. an antibody) "specifically binding"/that "specifically binds" to a second molecule/molecular group (e.g. an antigen of interest), this means that the first molecule/molecular group (in this example the antibody) binds to said second molecule/molecular group (in this example the antigen of interest) with an affinity that is at least ten-fold greater than its affinity for other molecules/molecular groups, in particular other molecule/molecular group in the human body (in this example at least ten-fold greater than its affinity for binding to non-specific antigens (e.g., BSA, casein) other than said antigen of interest (or closely related antigens)). In a preferred embodiment, a first molecule/molecular group (e.g. an antibody) that "specifically binds" to a second molecule/molecular group (e.g. an antigen of interest) binds to said antigen with an affinity that is at least 100-fold greater than its affinity for other molecules/molecular groups, in particular other molecule/molecular group in the human body (in this example at least 100-fold greater than its affinity for binding to non-specific antigens other than said antigen of interest (or closely related antigens)). Typically said binding will be determined under physiological conditions. A first molecule/molecular group that "specifically binds" to a second molecule/molecular group may bind to that second molecule/molecular group with a KD of $1x10^{-7}$ M or stronger. An antibody that "specifically binds" to an antigen of interest may bind to that antigen with a KD of $1x10^{-7}$ M or stronger. In some embodiments, said KD value is measured by kinetic measurements by biolayer interferometry at 25°C and 1000 rpm in KB Buffer (PBS + 0.1 % Tween-20 + 1% BSA).

Embodiment 150: The compound according to any one of embodiments 5 to 149, wherein said Ligand unit (resp. said antibody, i.e. the antibody that is included as Ligand unit in said compound) is an antibody against an antigen that is present on the surface of a target cell or an antigen-binding fragment of such an antibody.

An antibody "against" a certain antigen is an antibody with an antigen-binding site that binds to said antigen. If an antibody binds to an antigen can e.g. be determined by testing in an immunofluorescence experiment with cultured cells whether the antibody binds to cells that express the antigen at their cell surface.

Embodiment 151: The compound according to embodiment 5 to 150, wherein said antigen is more abundant on the surface of said target cell than on the surface of other cell types.

The abundance of a surface antigen on a cell type can be determined by standard methods known to a skilled person, e.g. flow cytometry (e.g. by exposing a cell of said cell type to the antibody of interest, subsequently staining with a fluorescently labelled secondary antibody directed against the antibody of interest, and detection of fluorescent label by flow cytometry).

Embodiment 152: The compound according to any one of embodiments 5 to 151, wherein said antigen is present on the surface of said target cell, but substantially not on the surface of other cell types.

As used herein, an antigen that is "present on the surface of said target cell, but substantially not on the surface of other cell types" is sufficiently abundant at the surface of the target cell to allow for recruitment of a compound comprising an antibody against said antigen under physiological conditions. In contrast, abundance of said antigen at the surface of other cell types is so low that recruitment of said compound under physiological conditions is barely above background binding.

Embodiment 153: The compound according to any one of embodiments 5 to 152, wherein said antigen is present on the surface of said target cell, but not on the surface of other cell types.

As used herein, an antigen that is "present on the surface of said target cell, but not on the surface of other cell types" is sufficiently abundant at the surface of the target cell to allow for

recruitment of a compound comprising an antibody against said antigen under physiological conditions. In contrast, abundance of said antigen at the surface of other cell types is so low that recruitment of said compound under physiological conditions is not above background binding.

Embodiment 154: The compound according to any one of embodiments 5 to 153, wherein said binding of said Ligand unit (resp. said antibody, i.e. the antibody that is included as Ligand unit in said compound) to said antigen allows to recruit the compound specifically to said target cell.

The term "allows to recruit the antibody-drug conjugate specifically to said target cell" means that the compound is recruited to said target cell under physiological conditions with an efficiency that is at least 10 times higher, preferably at least 100 times higher, than the recruitment to other cell types (i.e. to other cell types to which said compound may be exposed in the body during administration of said compound).

Embodiment 155: The compound according to any one of embodiments 5 to 154, wherein said antigen is selected from the group consisting of a tumor antigen and an immune cell antigen.

Embodiment 156: The compound according to any one of embodiments 5 to 155, wherein said antigen that is present on the surface of said target cell is a tumor antigen.

Embodiment 157: The compound according to any one of embodiments 5 to 156, wherein said Ligand unit/said antibody is capable of specifically binding to an antigen selected from the group consisting of a tumor antigen and an immune cell antigen.

Embodiment 158: The compound according to any one of embodiments 5 to 157, wherein said Ligand unit/said antibody is capable of specifically binding to a tumor antigen.

As used herein, a "tumor antigen" is, in its broadest sense, an antigen that allows recruitment of a compound according to the present disclosure to the site of a tumor. Upon recruitment of said compound, a therapeutic action or diagnostic action (e.g. labelling of the tumor site) can be achieved. The tumor antigen may either be an antigen that is present on the surface of the tumor cells or an antigen associated with the tumor microenvironment.

The term "tumor", as used herein, refers to an abnormal cell mass formed by neoplastic cell growth. A tumor can be benign or malignant. Preferably, in the present disclosure the term "tumor" refers to a malignant tumor. The tumor can for example be, but is not limited to, a tumor present in myeloma, hematological cancers such as leukemias and lymphomas (such as B cell lymphoma, T cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma), sarcoma, lung cancer, liver cancer, or bladder cancer (see Rosenberg, Ann. Rev. Med. (1996), vol. 47, p. 481-491).

As used herein, the term "cancer" refers to a malignant neoplasm. Cancer can include a hematological cancer or a solid tumor. For example, the cancer can be a leukemia (e.g., acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) or lymphoma (e.g., non-Hodgkin lymphoma), lung cancer (e.g., non-small cell lung cancer; NSCLC), ovarian cancer, or breast cancer. Preferably, in the present disclosure the term "cancer" refers to a solid malignant tumor.

Non-limiting examples of tumor antigens are EGFR, c-MET and CEA5 (Carcinoembryonic antigen 5).

Insofar as the designations of antigens indicated in the present disclosure are gene designations, these designations refer to the protein(s) encoded by said gene.

Sources for information on cell surface expression and methods to identify and verify tumor antigens are known to a skilled person and described in the literature (see e.g. Bornstein, AAPS J. (2015), vol. 17(3), p. 525–534; Bander, Methods Mol Biol (2013), vol. 1045, p. 29-40; Antibody-Drug Conjugates: Fundamentals, Drug Development, and Clinical Outcomes to Target Cancer", 1st edition (2016), editors Olivier and Hurvitz, publisher John Wiley & Sons, Inc. (U.S.); Vigneron et al., Cancer Immun. (2013), vol. 13, p. 15; Hong et al., BMC Syst Biol. (2018), vol. 12 (Suppl 2), p. 17; de Souza et al., Cancer Immun. (2012), vol. 12, p. 15; Immune Epitope Database and Analysis Resource (https://www.iedb.org); Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle); OASIS Database (http://oasis-genomics.org/)).

Embodiment 159: The compound according to any one of embodiments 155 to 158, wherein said tumor antigen is an antigen that is present on the surface of a tumor cell.

Embodiment 160: The compound according to any one of embodiments 155 to 159, wherein the term "tumor antigen" indicates an antigen that is present at the cell surface of a tumor cell and allows for distinction of the tumor cell over other cell types.

Embodiment 161: The compound according to any one of embodiments 155 to 160, wherein said tumor antigen is part of a molecule (e.g. a protein) that is expressed by a tumor cell and accessible from the extracellular environment. A tumor antigen may differ (i.e. qualitatively differ) from its counterpart in corresponding non-tumor cells (e.g., where the molecule is a protein by one or more amino acid residues). Alternatively, the tumor antigen may be identical to its counterpart in corresponding non-tumor cells, but present on the surface of the tumor cells at a higher level than on the surface of corresponding non-tumor cells. For example, the tumor antigen may be present only on the surface of the tumor cells, but not on the surface of non-tumor cells, or the tumor antigen may be present on the surface of tumor cells at a higher level (e.g. at least 5-fold higher, preferably at least 100-fold higher) than on the surface of non-tumor cells. In an embodiment, the tumor antigen is present on the surface of tumor cells at a level that is at least 1000-fold higher than on the surface of non-tumor cells.

Embodiment 162: The compound according to any one of embodiments 155 to 161, wherein the tumor antigen is present only on the surface of the tumor cells, but not on the surface of non-tumor cells.

Embodiment 163: The compound according to any one of embodiments 155 to 162, wherein the tumor antigen is present on the surface of tumor cells at a higher level (e.g. at least 5-fold higher, preferably at least 100-fold higher) than on the surface of non-tumor cells.

Embodiment 164: The compound according to any one of embodiments 155 to 163, wherein the tumor antigen is present on the surface of tumor cells at a level that is at least 1000-fold higher than on the surface of non-tumor cells.

Embodiment 165: The compound according to any one of embodiments 155 to 164, wherein the tumor to which said tumor antigen relates is a cancer (i.e. the tumor antigen that is present on the surface of a tumor cell is present on a cancer cell).

Embodiment 166: The compound according to any one of embodiments 5 to 165, wherein the Ligand unit is an antibody or an antigen-binding fragment of an antibody.

Embodiment 167: The compound according to any one of embodiments 5 to 166, wherein the Ligand is an antibody which specifically binds to an antigen that is present on the surface of a target cell and thus recruits said compound (of which that Ligand unit is a part) to said target cell.

Embodiment 168: The compound according to any one of embodiments 1 to 167, wherein each compound comprises one antibody. As the skilled person understands, a according to the present disclosure comprises one antibody which comprises one antibody may comprise e.g. more than one Linker unit and more than one Functional agent.

Embodiment 169: The compound according to any one of embodiments 5 to 168, wherein the Ligand unit comprises additional molecular group(s) in addition to the part of the molecule that is directly involved in the binding to the target site.

Embodiment 170: The compound according to any one of embodiments 5 to 168, wherein the Ligand unit does not comprise additional molecular groups in addition to the part of the molecule that is directly involved in the binding to the target site.

Embodiment 171: The compound according to any one of embodiments 5 to 170, wherein said Ligand unit/said antibody (i.e. said antibody that is included as targeting moiety in the compound) is capable of specifically binding to an immune cell antigen.

Embodiment 172: The compound according to any one of embodiments 155 to 171, wherein said immune cell antigen is

- an antigen present on the surface of an immune cell,
- an antigen which is a molecule that is secreted by an immune cell, or
- an antigen which is a molecule that interacts with a receptor on an immune cell.

Embodiment 173: The compound according to any one of embodiments 155 to 172, wherein said immune cell antigen is an antigen present on the surface of an immune cell.

Embodiment 174: The compound according to any one of embodiments 1 to 173, wherein said antigen is an immune cell antigen that is present on the surface of an immune cell.

Embodiment 175: The compound according to any one of embodiments 155 to 174, wherein said immune cell is a B cell, a T cell or a dendritic cell. Preferably, said immune cell is a T cell.

For example, an immune cell antigen may be e.g. CD80, IL-4 or interferon receptor.

Embodiment 176: The compound according to any one of embodiments 155 to 175, wherein binding of said Ligand unit (resp. said antibody that is included as Ligand unit in the compound) to said immune cell antigen has an immunostimulatory or immunosuppressive effect.

Embodiment 177: The compound according to any one of embodiments 5 to 176, wherein said antibody is an antibody against an antigen that is present on the surface of a target cell.

Embodiment 178: The compound according to any one of embodiments 5 to 177, wherein said antigen-binding fragment is an antigen-binding fragment of an antibody against an antigen that is present on the surface of a target cell.

Embodiment 179: The compound according to any one of embodiments 5 to 178, wherein said Ligand unit/said antibody (i.e. the antibody that is included as Ligand unit in said compound) has a first and a second antigen-binding site.

Embodiment 180: The compound according to embodiment 179, wherein said first and said second antigen-binding site are capable of binding to different antigens.

Embodiment 181: The compound according to any one of embodiments 179 to 180, wherein said first antigen-binding site is capable of specifically binding to a tumor antigen and said second antigen-binding site is capable of specifically binding to a tumor antigen.

Embodiment 182: The compound according to any one of embodiments 5 to 181, wherein said Ligand unit (L) is a monoclonal antibody.

Embodiment 183: The compound according to any one of embodiments 5 to 182, wherein said Ligand unit is a molecular group that specifically binds to a target molecule or fragment thereof.

Embodiment 184: The compound according to embodiment 183, wherein said target molecule is a biomolecule.

Embodiment 185: The compound according to any one of embodiments 183 to 184, wherein said target molecule is a receptor at the surface of a cell.

Embodiment 186: The compound according to any one of embodiments 183 to 185, wherein said target molecule is an antigen that is present on the surface of a target cell.

Embodiment 187: The compound according to any one of embodiments 5 to 186, wherein said targeting moiety is capable of specifically binding to an antigen that is present on the surface of a target cell.

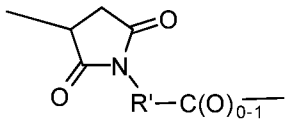
Embodiment 188: The compound according to any one of embodiments 1 to 187, wherein said Stretcher unit (A) is a molecular group that is covalently linked to the Ligand unit and the Iduronide unit (W) and thus forms a connection between these two molecular components of the compound.

Embodiment 189: The compound according to any one of embodiments 1 to 188, wherein said Stretcher unit (A) is a molecular group that covalently links the Ligand unit (L) to the Iduronide unit (W).

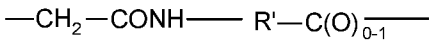
Embodiment 190: The compound according to any one of embodiments 1 to 189, wherein the Stretcher unit forms a bond with a sulfur atom of the Ligand unit.

Embodiment 191: The compound according to embodiment 190, wherein the sulfur atom is derived from a sulfhydryl group of the Ligand unit.

Embodiment 192: The compound according to any one of embodiments 1 to 191, wherein the Stretcher unit has a structure according to a formula selected from the group consisting of the following formulas:



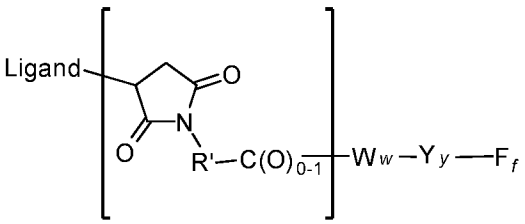
(Formula 33)



(Formula 34),

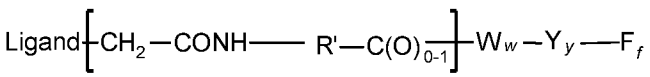
wherein R' is a direct bond or selected from the group consisting of C_1-C_{10} alkylene, C_3-C_8 carbocyclo, –O–(C_1-C_8 alkyl), arylene, C_1-C_{10} alkylene-arylene-, arylene-C_1-C_{10} alkylene, C_1-C_{10} alkylene (C_3-C_8 carbocyclo), (C_3-C_8 carbocyclo) –C_1-C_{10} alkylene-, C_3-C_8 heterocyclo-, –C_1-C_{10} alkylene-(C_3-C_8 heterocyclo)-, (C_3-C_8 heterocyclo) –C_1-C_{10} alkylene, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, and –$(CH_2CH_2O)_r$–CH_2–CH_2–; and r is an integer ranging from 1-10.

If the Stretcher unit has a structure according to Formula 33, then the Stretcher unit will typically be linked to the Ligand unit and the Iduronide unit(s) in the following orientation (and analogously in cases where there are multiple copies of individual components):



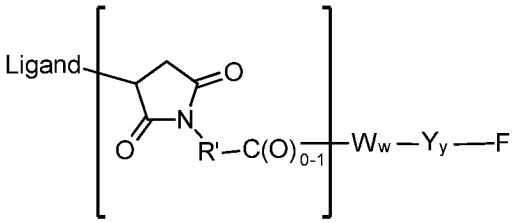
(Formula 35).

If the Stretcher unit has a structure according to Formula 34, then the Stretcher unit will typically be linked to the Ligand unit and the Iduronide unit(s) in the following orientation (and analogously in cases where there are multiple copies of individual components):

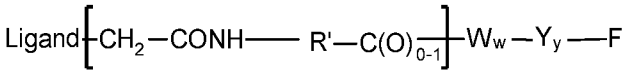


(Formula 36).

Embodiment 193: The compound according to any one of embodiments 1 to 192, wherein the Stretcher unit has a structure as shown between the square brackets of one of the formulas depicted below:



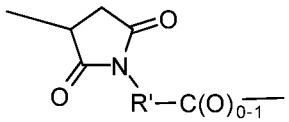
(Formula 37)



(Formula 38),

wherein R' is a direct bond or selected from C_1-C_{10} alkylene, C_3-C_8 carbocyclo, –O–(C_1-C_8 alkyl), arylene, C_1-C_{10} alkylene-arylene-, arylene-C_1-C_{10} alkylene, C_1-C_{10} alkylene (C_3-C_8 carbocyclo), (C_3-C_8 carbocyclo) –C_1-C_{10} alkylene-, C_3-C_8 heterocyclo-, –C_1-C_{10} alkylene-(C_3-C_8 heterocyclo)-, (C_3-C_8 heterocyclo) –C_1-C_{10} alkylene, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, and –$(CH_2CH_2O)_r$–CH_2–CH_2–; and r is an integer ranging from 1-10.

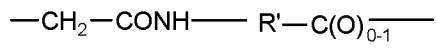
Embodiment 194: The compound according to any one of embodiments 1 to 193, wherein the Stretcher unit has a structure according to the following formula:



(Formula 33),

wherein R' is a direct bond or selected from the group consisting of C_1-C_{10} alkylene, C_3-C_8 carbocyclo, –O–(C_1-C_8 alkyl), arylene, C_1-C_{10} alkylene-arylene-, arylene-C_1-C_{10} alkylene, C_1-C_{10} alkylene (C_3-C_8 carbocyclo), (C_3-C_8 carbocyclo) –C_1-C_{10} alkylene-, C_3-C_8 heterocyclo-, –C_1-C_{10} alkylene-(C_3-C_8 heterocyclo)-, (C_3-C_8 heterocyclo) –C_1-C_{10} alkylene, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, and –$(CH_2CH_2O)_r$–CH_2–CH_2–; and r is an integer ranging from 1-10.

Embodiment 195: The compound according to any one of embodiments 1 to 193, wherein the Stretcher unit has a structure according to the following formula:



(Formula 34),

wherein R' is a direct bond or selected from the group consisting of C_1-C_{10} alkylene, C_3-C_8 carbocyclo, –O–(C_1-C_8 alkyl), arylene, C_1-C_{10} alkylene-arylene-, arylene-C_1-C_{10} alkylene, C_1-C_{10} alkylene (C_3-C_8 carbocyclo), (C_3-C_8 carbocyclo) –C_1-C_{10} alkylene-, C_3-C_8 heterocyclo-, –C_1-C_{10} alkylene-(C_3-C_8 heterocyclo)-, (C_3-C_8 heterocyclo) –C_1-C_{10} alkylene, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, and –$(CH_2CH_2O)_r$–CH_2–CH_2–; and r is an integer ranging from 1-10.

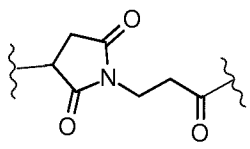
Embodiment 196: The compound according to any one of embodiments 1 to 195, wherein R' is –$(CH_2)_2$–.

Embodiment 197: The compound according to any one of embodiments 1 to 195, wherein R' is –$(CH_2)_5$–.

Embodiment 198: The compound according to any one of embodiments 1 to 195, wherein R' is –$(CH_2CH_2O)_r$–CH_2–; and r is 2.

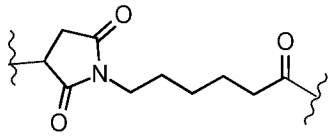
Embodiment 199: The compound according to any one of embodiments 1 to 195, wherein R' is –(CH_2CH_2)CONHCH$_2$–.

Embodiment 200: The compound according to any one of embodiments 1 to 196, wherein the Stretcher unit has a structure according to the following formula:



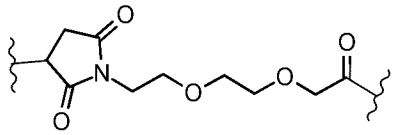
(Formula 12).

Embodiment 201: The compound according to any one of embodiments 1 to 195 or 197, wherein the Stretcher unit has a structure according to the following formula:



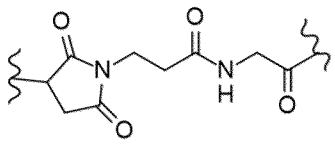
(Formula 13).

Embodiment 202: The compound according to any one of embodiments 1 to 195 or 198, wherein the Stretcher unit has a structure according to the following formula:



(Formula 14).

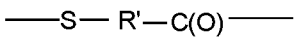
Embodiment 203: The compound according to any one of embodiments 1 to 195 or 199, wherein the Stretcher unit has a structure according to the following formula:



(Formula 39).

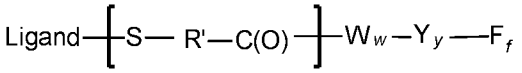
Embodiment 204: The compound according to any one of embodiments 1 to 203, wherein the Stretcher unit is linked to the Ligand unit via a disulfide bond between a sulfur atom of the Ligand unit and a sulfur atom of the Stretcher unit.

Embodiment 205: The compound according to any one of embodiments 1 to 193 or 195 to 199, wherein the Stretcher unit has a structure according to the following formula:



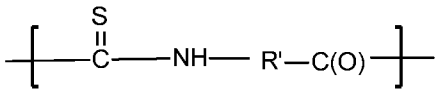
(Formula 40).

If the Stretcher unit has a structure according to Formula 40, then the Stretcher unit will typically be linked to the Ligand unit and the Iduronide unit(s) in the following orientation (and analogously in cases where there are multiple copies of individual components):



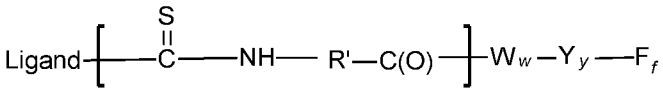
(Formula 41).

Embodiment 206: The compound according to any one of embodiments 1 to 193 or 195 to 199 or 203 to 205, wherein the Stretcher unit has a structure according to the following formula:



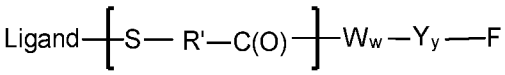
(Formula 42).

If the Stretcher unit has a structure according to Formula 42, then the Stretcher unit will typically be linked to the Ligand unit and the Iduronide unit(s) in the following orientation (and analogously in cases where there are multiple copies of individual components):

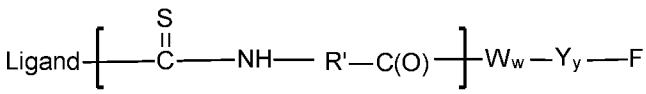


(Formula 43).

Embodiment 207: The compound according to any one of embodiments 1 to 193 or 195 to 199 or 203 to 205, wherein the Stretcher unit has a structure as shown between the square brackets of one of the formulas depicted below:



(Formula 44)



(Formula 45)

wherein R' is a direct bond or selected from C_1-C_{10} alkylene, C_3-C_8 carbocyclo, –O–(C_1-C_8 alkyl), arylene, C_1-C_{10} alkylene-arylene-, arylene-C_1-C_{10} alkylene, C_1-C_{10} alkylene (C_3-C_8 carbocyclo), (C_3-C_8 carbocyclo) –C_1-C_{10} alkylene-, C_3-C_8 heterocyclo-, –C_1-C_{10} alkylene-(C_3-C_8 heterocyclo)-, (C_3-C_8 heterocyclo) –C_1-C_{10} alkylene, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, and –$(CH_2CH_2O)_r$–CH_2–CH_2–; and r is an integer ranging from 1-10.

Embodiment 208: The compound according to any one of embodiments 1 to 191, wherein the Stretcher unit has a structure according to the following formula:

(Formula 46),

wherein s is an integer from 1 to 5.

Embodiment 209: The compound according to any one of embodiments 1 to 191, wherein the Stretcher unit has a structure according to the following formula:

(Formula 47).

Embodiment 210: The compound according to any one of embodiments 1 to 209, wherein the presence of said Stretcher unit in said compound does not interfere with the function of said compound.

Embodiment 211: The compound according to any one of embodiments 1 to 210, wherein the presence of said Stretcher unit in said compound does not interfere with the specific binding properties of the Ligand unit.

Embodiment 212: The compound according to any one of embodiments 1 to 211, wherein the presence of said Stretcher unit in said compound does not interfere with the function of the Functional agent.

Embodiment 213: The compound according to any one of embodiments 1 to 212, wherein the presence of said Stretcher unit in said compound does not interfere with the binding/targeting function of the Ligand unit, the iduronidase-mediated cleavage of the Iduronide unit and the activity of the Functional agent.

Embodiment 214: The compound according to any one of embodiments 1 to 213, wherein there is one Stretcher unit or more than one Stretcher unit within a Linker unit.

Embodiment 215: The compound according to any one of embodiments 1 to 214, wherein there is one Stretcher unit or more than one Stretcher unit within a Linker-Functional agent unit.

Embodiment 216: The compound according to any one of embodiments 1 to 215, wherein there is more than one Linker-Functional agent unit in said compound.

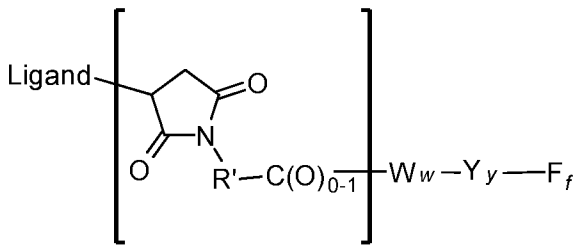
Embodiment 217: The compound according to any one of embodiments 1 to 216, wherein all Stretcher units present in said compound have an identical structure.

Embodiment 218: The compound according to any one of embodiments 1 to 217, wherein, if a Stretcher unit is present, there is only one Stretcher unit per Linker-Functional agent unit.

Embodiment 219: The compound according to any one of embodiments 1 to 218, wherein all Stretcher units within said compound have the same chemical structure.

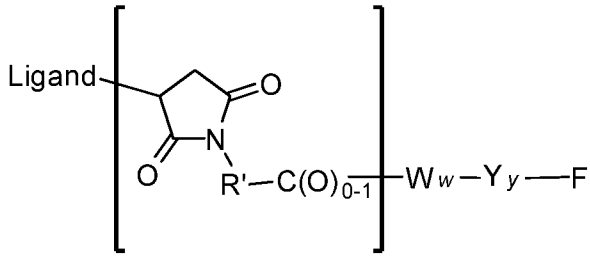
Embodiment 220: The compound according to any one of embodiments 1 to 219, wherein at each occurrence of the Stretcher unit, the structure of the Stretcher unit A is independently selected.

Embodiment 221: The compound according to any one of embodiments 1 to 194 or 196 to 204 or 210 to 220, wherein the compound has a structure according to the following formula:



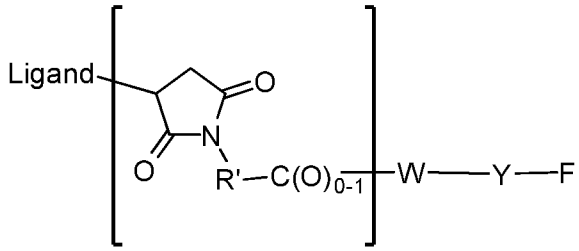
(Formula 35).

Embodiment 222: The compound according to any one of embodiments 1 to 194 or 196 to 204 or 210 to 220, wherein the compound has a structure according to the following formula:



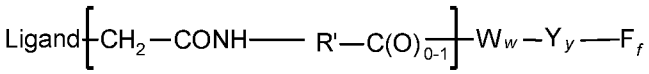
(Formula 37).

Embodiment 223: The compound according to any one of embodiments 1 to 194 or 196 to 204 or 210 to 220, wherein the compound has a structure according to the following formula:



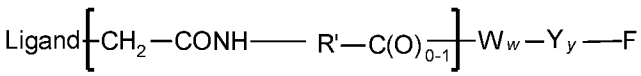
(Formula 48).

Embodiment 224: The compound according to any one of embodiments 1 to 193 or 195 to 199 or 204 to 207 or 210 to 220, wherein the compound has a structure according to the following formula:



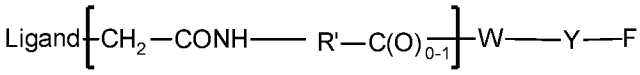
(Formula 36).

Embodiment 225: The compound according to any one of embodiments 1 to 193 or 195 to 199 or 204 to 207 or 210 to 220, wherein the compound has a structure according to the following formula:



(Formula 38).

Embodiment 226: The compound according to any one of embodiments 1 to 193 or 195 to 199 or 204 to 207 or 210 to 220, wherein the compound has a structure according to the following formula:



(Formula 49).

Embodiment 227: The compound according to any one of embodiments 1 to 226, wherein the Spacer unit(s), when present, link(s) said Iduronide unit to said Functional agent.

Embodiment 228: The compound according to any one of embodiments 1 to 227, wherein the Spacer unit (Y), when present in said compound, is a molecular group that is covalently linked to the Iduronide unit (W) and the Functional agent and thus forms a connection between these two molecular components of said compound.

Embodiment 229: The compound according to any one of embodiments 1 to 228, wherein the Spacer unit(s) is a self-immolative spacer.

Embodiment 230: The compound according to any one of embodiments 1 to 229, wherein said self-immolative spacer is a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a normally stable tripartite molecule.

Embodiment 231: The compound according to any one of embodiments 1 to 230, wherein said self-immolative spacer spontaneously separates from the second chemical moiety if its bond to the first moiety is cleaved.

Embodiment 232: The compound according to any one of embodiments 1 to 231, wherein said self-immolative spacer is a chemical moiety that provides a covalent link between said Iduronide unit and said Functional agent.

Embodiment 233: The compound according to any one of embodiments 1 to 232, wherein, upon cleavage of the bond between said self-immolative spacer and said Iduronide unit, said self-immolative spacer decomposes in such a way that the covalent bond between said Spacer unit and said Functional agent is disrupted.

Embodiment 234: The compound according to any one of embodiments 1 to 233, wherein, upon cleavage of the bond between said self-immolative spacer and said Functional agent, said self-immolative spacer decomposes in such a way that the covalent bond between said Spacer unit and said Iduronide unit is disrupted.

Embodiment 235: The compound according to any one of embodiments 1 to 234, wherein said self-immolative spacer is a bi-functional chemical moiety that is capable of covalently linking the Iduronide unit and the Functional agent.

Embodiment 236: The compound according to any one of embodiments 1 to 235, wherein said self-immolative spacer spontaneously separates from the Functional agent and/or the Iduronide unit if the covalent bond between said self-immolative spacer and said Functional agent or the covalent bond between said self-immolative spacer and said Iduronide unit is cleaved.

Embodiment 237: The compound according to any one of embodiments 1 to 236, wherein said Spacer unit forms a covalent connection between the Iduronide unit and the Functional agent.

Embodiment 238: The compound according to any one of embodiments 1 to 237, wherein said Spacer unit is linked to the Iduronide unit via the methylene carbon atom of the self-immolative group.

Embodiment 239: The compound according to any one of embodiments 1 to 238, wherein said Spacer unit is linked directly to the Functional agent via a carbonate, carbamate or ether group.

Embodiment 240: The compound according to any one of embodiments 1 to 239, wherein said Spacer unit comprises a carbonate group.

Embodiment 241: The compound according to any one of embodiments 1 to 240, wherein said Spacer unit is an aromatic compound that is electronically similar to the meta-aminobenzyl alcohol group.

Embodiment 242: The compound according to any one of embodiments 1 to 241, wherein said Spacer unit is selected from the group consisting of a 2-aminoimidazol-5- methanol derivative, an ortho-aminobenzylacetal and a para-aminobenzylacetal.

Embodiment 243: The compound according to any one of embodiments 1 to 241, wherein said Spacer unit is selected from the group consisting of an ortho-aminobenzylacetal and a para-aminobenzylacetal.

Embodiment 244: The compound according to any one of embodiments 1 to 243, wherein said Spacer unit undergoes cyclization upon amide bond hydrolysis.

Embodiment 245: The compound according to any one of embodiments 1 to 244, wherein said Spacer unit is selected from the group consisting of a substituted or unsubstituted 4-aminobutyric acid amide, a substituted bicyclo 2.2.1 ring system, a substituted bicyclo2.2.2 ring system and a 2-aminophenylpropionic acid amide.

Embodiment 246: The compound according to any one of embodiments 1 to 245, wherein said Spacer unit is an amine-containing drug that is substituted at the a-position of glycine.

Embodiment 247: The compound according to any one of embodiments 1 to 246, wherein the Spacer unit is linked to the Iduronide unit via a methylene carbon atom of the self-immolative group.

Embodiment 248: The compound according to any one of embodiments 1 to 245, wherein the Spacer unit is a meta-aminobenzyl alcohol unit whose phenylene portion is substituted with Q_m, wherein Q is selected from the group consisting of –(C_1-C_8 alkyl), –O–(C_1-C_8 alkyl), –halogen, –nitro and –cyano; and *m* is 0, 1, 2, 3 or 4.

Embodiment 249: The compound according to any one of embodiments 1 to 245, wherein the Spacer unit is a para-aminobenzyl alcohol unit whose phenylene portion is substituted with Q_m, wherein Q is selected from the group consisting of –(C_1-C_8 alkyl), –O–(C_1-C_8 alkyl), –halogen, –nitro and –cyano; and *m* is 0, 1, 2, 3 or 4.

Embodiment 250: The compound according to any one of embodiments 248 or 249, wherein *m* is 0, 1 or 2.

Embodiment 251: The compound according to any one of embodiments 248 or 249, wherein *m* is 0 or 1.

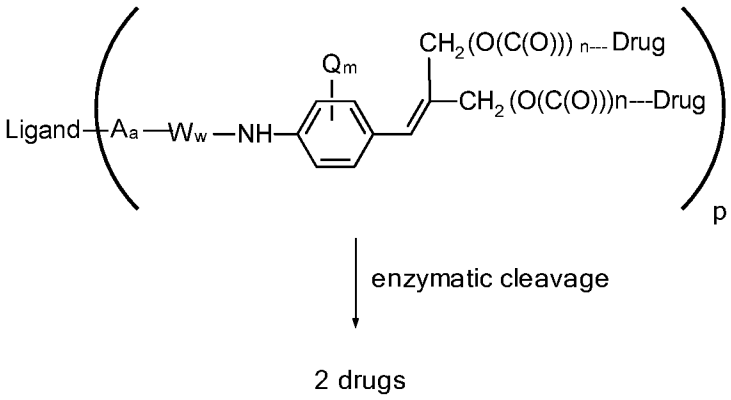
Embodiment 252: The compound according to any one of embodiments 248 or 249, wherein *m* is 1.

Embodiment 253: The compound according to any one of embodiments 248 or 249, wherein *m* is 0.

Embodiment 254: The compound according to any one of embodiments 1 to 238 or 240 or 247, wherein the Spacer unit is a carbonate group.

Embodiment 255: The compound according to any one of embodiments 1 to 254, wherein the Spacer unit is a branched bis(hydroxymethyl)styrene (BHMS) unit.

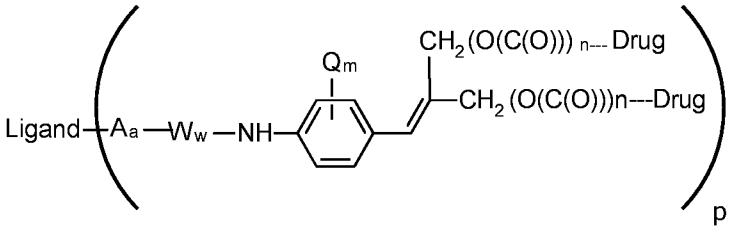
A branched bis(hydroxymethyl)styrene (BHMS) unit as Spacer unit is depicted in the following scheme:



wherein Q is selected from the group consisting of –(C_1-C_8 alkyl), –O–(C_1-C_8 alkyl). –halogen, –nitro and –cyano; *m* is 0, 1, 2, 3 or 4; *n* is 0 or 1; and p is from 1 to 20.

Such a Spacer unit can be used to incorporate and release multiple drugs.

Embodiment 256: The compound according to any one of embodiments 1 to 255, wherein the Spacer unit is as shown in the formula below between "Ww" and "Drug":



(Formula 50).

Embodiment 257: The compound according to any one of embodiments 1 to 256, wherein the Spacer unit does not interfere with the function of the compound.

Embodiment 258: The compound according to any one of embodiments 1 to 257, wherein the Spacer unit does not interfere with the binding/targeting function of the Ligand unit, the iduronidase-mediated cleavage of the Iduronide unit and the activity of the Functional agent.

Embodiment 259: The compound according to any one of embodiments 1 to 258, wherein said Spacer unit does not interfere with the binding/targeting function of the Ligand unit, the iduronidase-mediated cleavage of the Iduronide unit and the activity of the Functional agent.

Embodiment 260: The compound according to any one of embodiments 1 to 259, wherein the presence of said Spacer unit in said compound does not interfere with the function of said compound.

Embodiment 261: The compound according to any one of embodiments 1 to 260, wherein the presence of said Spacer unit in said compound does not interfere with the specific binding properties of the Ligand unit.

Embodiment 262: The compound according to any one of embodiments 1 to 261, wherein the presence of said Spacer unit in said compound does not interfere with the function of the Functional agent.

Embodiment 263: The compound according to any one of embodiments 1 to 262, wherein the presence of said Spacer unit in said compound does not interfere with the binding/targeting

function of the Ligand unit, the iduronidase-mediated cleavage of the Iduronide unit and the activity of the Functional agent.

Embodiment 264: The compound according to any one of embodiments 1 to 263, wherein there is one Spacer unit or more than one Spacer unit within a Linker unit (resp. within a Linker-Functional agent unit).

Embodiment 265: The compound according to any one of embodiments 1 to 264, wherein there is one Spacer unit or more than one Spacer unit within each Linker unit of said compound.

Embodiment 266: The compound according to any one of embodiments 1 to 265, wherein there is one Spacer unit within each Linker unit of said compound.

Embodiment 267: The compound according to any one of embodiments 1 to 266, wherein there is more than one Spacer unit within each Linker unit of said compound.

Embodiment 268: The compound according to any one of embodiments 1 to 267, wherein there is one Spacer unit or more than one Spacer unit within each Linker-Functional agent unit of said compound.

Embodiment 269: The compound according to any one of embodiments 1 to 266 or 268, wherein there is one Spacer unit within each Linker-Functional agent unit of said compound

Embodiment 270: The compound according to any one of embodiments 1 to 265 or 267 to 266, wherein there is more than one Spacer unit within each Linker-Functional agent unit of said compound

Embodiment 271: The compound according to any one of embodiments 1 to 270, wherein there is more than one Linker-Functional agent unit in said compound.

Embodiment 272: The compound according to any one of embodiments 1 to 271, wherein all Spacer units present in said compound have an identical structure.

Embodiment 273: The compound according to any one of embodiments 1 to 266 or 268 to 267 or 270, wherein, if a Spacer unit is present, there is only one Spacer unit per Linker unit.

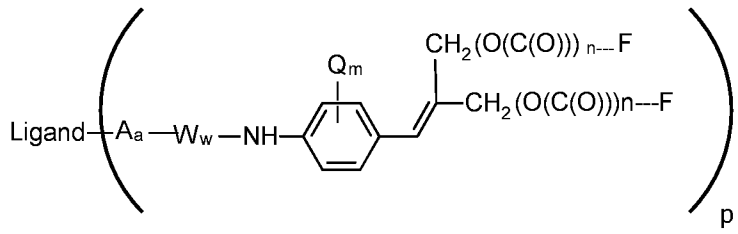
Embodiment 274: The compound according to any one of embodiments 1 to 266 or 268 to 267 or 270 to 271, wherein, if a Spacer unit is present, there is only one Spacer unit per Linker-Functional agent unit.

Embodiment 275: The compound according to any one of embodiments 1 to 274, wherein all Spacer units within said compound have the same chemical structure.

Embodiment 276: The compound according to any one of embodiments 1 to 275, wherein at each occurrence of the Spacer unit, the structure of the Spacer unit Y is independently selected.

Embodiment 277: The compound according to any one of embodiments 1 to 276, wherein more than one Linker-Functional agent unit is present in said compound.

Embodiment 278: The compound according to any one of embodiments 1 to 277, wherein said compound has a structure according to the following formula:



(Formula 51)

wherein Q is selected from the group consisting of –(C_1-C_8 alkyl), –O–(C_1-C_8 alkyl). –halogen, –nitro and –cyano; *m* is 0, 1, 2, 3 or 4; *n* is 0 or 1; and *p* is from 1 to 20.

Embodiment 279: The compound according to embodiment 278, wherein *m* is 0, 1 or 2.

Embodiment 280: The compound according to embodiment 278, wherein *m* is 0 or 1.

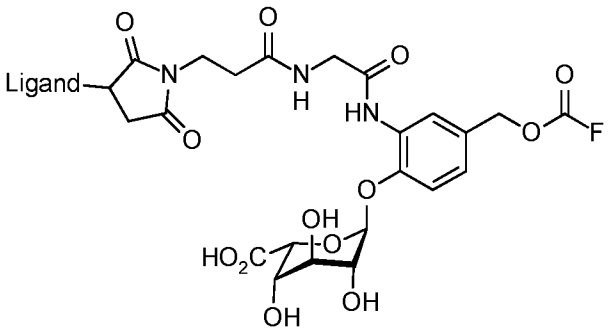
Embodiment 281: The compound according to embodiment 278, wherein *m* is 1.

Embodiment 282: The compound according to embodiment 278, wherein *m* is 0.

Embodiment 283: The compound according to any one of embodiments 278 to 282, wherein *n* is 1.

Embodiment 284: The compound according to any one of embodiments 278 to 282, wherein *n* is 0.

Embodiment 285: The compound according to any one of embodiments 1 to 284, wherein the compound has a structure according to the following formula:



(Formula 52)

Embodiment 286: The compound according to any one of embodiments 3 to 286, wherein said Functional agent comprises a protein, a peptide, a peptide mimetic, a nucleic acid, an oligonucleotide or a small molecule.

Embodiment 287: The compound according to any one of embodiments 3 to 286, wherein said Functional agent is a protein, a peptide, a peptide mimetic, a nucleic acid, an oligonucleotide or a small molecule.

Embodiment 288: The compound according to any one of embodiments 3 to 286, wherein said Functional agent comprises a protein.

Embodiment 289: The compound according to any one of embodiments 3 to 286, wherein said Functional agent is a protein.

Embodiment 290: The compound according to any one of embodiments 3 to 286, wherein said Functional agent comprises or is a small molecule.

Embodiment 291: The compound according to any one of embodiments 3 to 286, wherein said Functional agent comprises a small molecule.

Embodiment 292: The compound according to any one of embodiments 3 to 286, wherein said Functional agent is a small molecule.

Embodiment 293: The compound according to any one of embodiments 1 to 292, wherein a small molecule is a molecule with a molecular weight < 1000 Da.

Embodiment 294: The compound according to any one of embodiments 3 to 293, wherein said Functional agent has a molecular weight of at least 100 Da.

Embodiment 295: The compound according to any one of embodiments 3 to 293, wherein said Functional agent has a molecular weight of at least 500 Da.

Embodiment 296: The compound according to any one of embodiments 3 to 293, wherein said Functional agent has a molecular weight of at least 1 000 Da.

Embodiment 297: The compound according to any one of embodiments 3 to 293, wherein said Functional agent has a molecular weight of at least 2 000 Da.

Embodiment 298: The compound according to any one of embodiments 3 to 293, wherein said Functional agent has a molecular weight of at least 10 kDa.

Embodiment 299: The compound according to any one of embodiments 3 to 295, wherein said Functional agent has a molecular weight of up to 500 Da.

Embodiment 300: The compound according to any one of embodiments 3 to 296, wherein said Functional agent has a molecular weight of up to 1 000 Da.

Embodiment 301: The compound according to any one of embodiments 3 to 297, wherein said Functional agent has a molecular weight of up to 2 000 Da.

Embodiment 302: The compound according to any one of embodiments 3 to 298, wherein said Functional agent has a molecular weight of up to 10 kDa.

Embodiment 303: The compound according to any one of embodiments 3 to 302, wherein said Functional agent is a chemical entity which is capable of fulfilling a biological, chemical, therapeutic and/or diagnostic function in the human body.

Embodiment 304: The compound according to any one of embodiments 3 to 303, wherein said Functional agent is a payload that is a therapeutic agent.

Embodiment 305: The compound according to any one of embodiments 3 to 303, wherein said Functional agent is a therapeutic agent or a detectable label.

Embodiment 306: The compound according to any one of embodiments 3 to 305, wherein said Functional agent is a therapeutic agent. As the skilled person understands, in this case the Linker-Functional agent unit is a drug-linker conjugate.

Embodiment 307: The compound according to any one of embodiments 3 to 306, wherein said therapeutic agent is an agent that exerts an effect that is linked to a therapeutic benefit if administered to a patient.

Embodiment 308: The compound according to any one of embodiments 3 to 307, wherein said therapeutic agents is a cytotoxic agent, anti-inflammatory agent, immunostimulatory agent or immunosuppressive agent.

Embodiment 309: The compound according to any one of embodiments 3 to 308, wherein said Functional agent is a drug moiety.

Embodiment 310: The compound according to any one of embodiments 3 to 309, wherein said Functional agent is a cytotoxic agent.

Embodiment 311: The compound according to any one of embodiments 3 to 310, wherein said therapeutic agent is a cytotoxic agent.

Embodiment 312: The compound according to any one of embodiments 308 to 311, wherein said cytotoxic agent is a substance that is toxic to cells (i.e. causes cell death or destruction).

Embodiment 313: The compound according to any one of embodiments 308 to 312, wherein said cytotoxic agent is a small molecule, peptide or nucleic acid molecule.

Embodiment 314: The compound according to any one of embodiments 308 to 313, wherein said cytotoxic agents is an auristatin (e.g. auristatin E, MMAE (monomethyl auristatin E), MMAF (monomethyl auristatin F), dolastatin 10, dolastatin 15), maytansinoid (e.g. maytansin, DM1, DM2, DM3, DM4), tubulysin, exatecan, camptothecin, SN38, Dxd, exatecan, duocarmycin, CBI dimer, doxorubicin or diazepine (e.g. pyrrolobenzodiazepine or indolinobenzodiazepine).

Embodiment 315: The compound according to any one of embodiments 308 to 314, wherein said cytotoxic agent is a chemotherapeutic agent or a radioactive isotope.

Embodiment 316: The compound according to any one of embodiments 308 to 315, wherein said cytotoxic agent is a chemotherapeutic agent.

Methods for attaching each of these to a compound as according to the present disclosure are known in the art (see e.g. Singh and Erickson, 2009).

Embodiment 317: The compound according to any one of embodiments 308 to 315, wherein said cytotoxic agent is a radioisotope.

Embodiment 318: The compound according to any one of embodiments 315 or 317, wherein said radioactive isotope is selected from the group consisting of At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, P^{212}, Zr^{89} and radioactive isotopes of Lu.

Embodiment 319: The compound according to any one of embodiments 309 to 316 or 318, wherein said cytotoxic agent is selected from the group consisting of an inhibitor of microtubule formation, an EG5 inhibitor and a DNA damaging agent.

Embodiment 320: The compound according to embodiment 319, wherein said inhibitor of microtubule formation is an inhibitor that acts by inhibiting tubulin polymerization or microtubule assembly, and thus has anti-proliferative/toxic effects on cells.

Embodiment 321: The compound according to any one of embodiments 319 to 320, wherein said cytotoxic agent is an inhibitor of microtubule formation which is selected from the group consisting of an auristatin, a maytansinoid and tubulysin.

Embodiment 322: The compound according to embodiment 319, wherein said EG5 inhibitor is an inhibitor that inhibits the protein EG5, and thus is toxic to cells.

Embodiment 323: The compound according to any one of embodiments 319 or 322, wherein said cytotoxic agent is an EG5 inhibitor which is selected from the group consisting of ispinesib, filanesib, litronesib and K858.

Embodiment 324: The compound according to embodiment 319, wherein said DNA damaging agent is an agent that acts to damage cellular DNA (e.g. by inducing double-strand breaks, cross-linking specific sites of DNA or intercalating between DNA base pairs).

Embodiment 325: The compound according to any one of embodiments 319 or 324, wherein said cytotoxic agent is a DNA damaging agent which is selected from the group consisting of a topoisomerase I inhibitor, a topoisomerase II inhibitor and a DNA alkylating agent.

Embodiment 326: The compound according to embodiment 325, wherein said cytotoxic agent is a topoisomerase I inhibitor which is exatecan or a camptothecin.

Embodiment 327: The compound according to embodiment 325, wherein said cytotoxic agent is a topoismerase II inhibitor which is doxorubicin.

Embodiment 328: The compound according to embodiment 325, wherein said cytotoxic agent is a DNA alkylating agent which is selected from the group consisting of duocarmycin, a CBI dimer and a diazepine (preferably a pyrrolobenzodiazepine or indolinobenzodiazepine).

Embodiment 329: The compound according to any one of embodiments 309 to 316 or 319 to 328, wherein said cytotoxic agent is an exatecan or a duocarmycin.

Embodiment 330: The compound according to any one of embodiments 3 to 329, wherein said Functional agent is selected from the group consisting of auristatin, MMAE (monomethyl auristatin E), duocarmycin, CBI (Cyclopropanebenz[e]indoline) dimer, maytansin, pyrrolobenzodiazepine and indolinobenzodiazepine.

Embodiment 331: The compound according to any one of embodiments 3 to 330, wherein said Functional agent is selected from the group consisting of anthracycline, doxorubicin, methotrexate, an auristatin (preferably monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF)), a maytansine, a maytansinoid derivative (DMs), a calicheamicin, a duocarymycin, a pyrrolobenzodiazepine (PBD) dimer, a topoisomerase I inhibitor (e.g. camptothecins and camptothecin derivates) and a triptolide.

Embodiment 332: The compound according to any one of embodiments 3 to 331, wherein said Functional agent is selected from the group consisting of a topoisomerase I inhibitor (e.g. exatecan, camptothecin, SN38, Dxd or a variant thereof), a topoisomerase II inhibitor (e.g. doxorubicin or a variant thereof), a DNA alkylating agent (e.g. duocarmycin, a CBI dimer, a pyrrolobenzodiazepine or a variant thereof), a transcription inhibitor (e.g. triptolide, CDK inhibitors), a Bcl-xL inhibitor (e.g. clezutoclax), a tyrosine kinase inhibitor (e.g. neolymphostin, dasatinib or staurosporine), an immune-stimulating agent (e.g. a STING or TLR agonist), an HSP90 inhibitor (e.g. a geldanamycin derivate), a splicing inhibitor (e.g. a pladienolide), a translation inhibitor (e.g. psymberin), a proteasome inhibitor (e.g. a carmaphycin B analogue) and a PROTAC (e.g GNE-987).

Embodiment 333: The compound according to any one of embodiments 3 to 332, wherein said Functional agent is selected from a group consisting of a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, an amanitin, a pyrrolobenzodiazepine, an RNA polymerase inhibitor, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of the protein CRM1, a DPPIV inhibitor, a proteasome inhibitor, an inhibitor of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin

inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

Embodiment 334: The compound according to any one of embodiments 3 to 333, wherein said Functional agent is a maytansinoid, wherein the maytansinoid is N(2')- deacetyl-N(2')-(3-mercapto-l-oxopropyl)-maytansine (DM1), N(2')-deacetyl-N(2')-(4-mercapto-l-oxopentyl)-maytansine (DM3) or N(2')-deacetyl-N2-(4- mercapto-4-methyl- 1 -oxopentyl)-maytansine (DM4).

Embodiment 335: The compound according to any one of embodiments 3 to 334, wherein said Functional agent is selected from the group consisting of anthracycline, doxorubicin, methotrexate, an auristatin (preferably monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF)), a maytansine, a maytansinoid derivative (DM), a calicheamicin, a duocarymycin, a pyrrolobenzodiazepine (PBD) dimer, a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, an HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an amanitin, a pyrrolobenzodiazepine, an RNA polymerase inhibitor, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of CRM1, a DPPIV inhibitor, a proteasome inhibitor, an inhibitor of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

Embodiment 336: The compound according to any one of embodiments 3 to 335, wherein said Functional agent is the tubulin inhibitor monomethyl auristatin E (MMAE).

Embodiment 337: The compound according to any one of embodiments 1 to 336, wherein said compound comprises only one type of payload.

Embodiment 338: The compound according to any one of embodiments 1 to 336, wherein said compound comprises several types of payloads.

Embodiment 339: The compound according to any one of embodiments 1 to 338, wherein said compound comprises one Functional agent per Linker unit.

Embodiment 340: The compound according to any one of embodiments 1 to 339, wherein said compound comprises only one type of Functional agent.

Embodiment 341: The compound according to any one of embodiments 1 to 339, wherein said compound comprises up to two kinds of Functional agent.

Embodiment 342: The compound according to any one of embodiments 3 to 341, wherein said Functional agent is a cytotoxic agent that shows a higher cytotoxicity after cleavage of the Linker unit and release compared to the previous state when the cytotoxic agent is still covalently attached via the Linker unit Ligand unit.

Embodiment 343: The compound according to any one of embodiments 3 to 310 or 337 to 341, wherein said therapeutic agent is an anti-inflammatory agent.

Embodiment 344: The compound according to embodiment 309 to 310 or 337 to 341 or 343, wherein an anti-inflammatory agent is a substance that reduces inflammation.

Embodiment 345: The compound according to any one of embodiments 309 to 310 or 337 to 341 or 343 to 344, wherein said anti-inflammatory agent is a glucocorticoid receptor agonist.

Embodiment 346: The compound according to any one of embodiments 309 to 310 or 337 to 341 or 343 to 345, wherein said anti-inflammatory agent is a steroid or a non-steroidal anti-inflammatory agent.

Embodiment 347: The compound according to any one of embodiments 3 to 310 or 337 to 341, wherein said therapeutic agent is an immunostimulatory agent.

Embodiment 348: The compound according to any one of embodiments 3 to 310 or 337 to 341 or 347, wherein said immunostimulatory agent is a substance that enhances the development or maintenance of an immunologic response.

Embodiment 349: The compound according to any one of embodiments 3 to 310 or 337 to 341 or 347 to 348, wherein said immunostimulatory agent is an agonist of an immunostimulatory molecule or an antagonist of a molecule inhibiting an immunologic response.

Embodiment 350: The compound according to any one of embodiments 3 to 310 or 337 to 341, wherein the therapeutic agent is an immunosuppressive agent.

Embodiment 351: The compound according to any one of embodiments 3 to 310 or 337 to 341 or 350, wherein said immunosuppressive agent is an agent that inhibits the development or maintenance of an immunologic response.

Embodiment 352: The compound according to any one of embodiments 3 to 310 or 337 to 341 or 350 to 351, wherein said immunosuppressive agent is capable of causing the reduction of an undesired immune response as compared to the administration of a control molecule that does not include said immunosuppressive agent.

Embodiment 353: The compound according to any one of embodiments 3 to 304 or 306 or 337 to 341, wherein said Functional agent is a detectable label.

Embodiment 354: The compound according to any one of embodiments 3 to 304 or 306 or 337 to 341 or 353, wherein said detectable label is a molecule capable of detection (i.e. capable of being detected by methods known in the art).

Embodiment 355: The compound according to any one of embodiments 3 to 304 or 306 or 337 to 341 or 353 to 354, wherein said detectable label is a radioisotope, fluorophore, chromophore, enzyme, dye, metal ion, ligand (such as biotin, avidin, streptavidin or hapten) or quantum dot.

Embodiment 356: The compound according to any one of embodiments 3 to 304 or 306 or 337 to 341 or 353 to 355, wherein said detectable label is a radioisotope, fluorescent compound or enzyme.

Embodiment 357: The compound according to any one of embodiments 3 to 304 or 306 or 337 to 341 or 353 to 356, wherein said detectable label is selected from the group consisting of a cyanine dye, a sulfo-cyanine dye, an Alexa Fluor® dye (Molecular Probes/Thermo Fisher

Scientific), a DyLight® Fluor dye (Dyomics/Thermo Fisher Scientific), FluoProbes® dyes (Interchim), a Seta® dye (SETA BioMedicals) and an IRIS™ dyes (Cyanine Technologies).

Embodiment 358: The compound according to any one of embodiments 3 to 304 or 306 or 337 to 341 or 353 to 357, wherein said detectable label is a cyanine dye or sulfo-cyanine dye.

Embodiment 359: The compound according to any one of embodiments 3 to 304 or 306 or 337 to 341 or 353 to 358, wherein said detectable label is a cyanine dye selected from the group consisting of Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7.

Embodiment 360: The compound according to any one of embodiments 3 to 304 or 306 or 337 to 341 or 353 to 359, wherein said detectable label is a sulfo-cyanine dye selected from the group consisting of sulfo-Cy2, sulfo-Cy3, sulfo-Cy3B, sulfo-Cy3.5, sulfo-Cy5, sulfo-Cy5.5, sulfo-Cy7.

Embodiment 361: The compound according to any one of embodiments 3 to 360, wherein said compound comprises two different types of Functional agents.

As the skilled person understands, this designates that the compound comprises two different types of functional agent. In other words, the compound comprises a functional agent with a certain structure, and in addition a second functional agents with a different structure. For example, the compound may comprise one type of duocarmycin and one type of maytansine, but in addition no other type of drug moiety or no other type of functional agent. Or, in another example, the compound may comprise two different types of duocarmycins, but in addition no other type of drug moiety and no other type of functional agent. Each of these two different types of functional agent (in the first example a type of duocarmycine and a type of maytansine; in the second example the two different types of duocarmycines) may be present in the compound in one or more copies.

Embodiment 362: The compound according to any one of embodiments 3 to 360, wherein said compound comprises only one type of Functional agent. In other words, the compound comprises a functional agent with a certain structure, but not functional agents with a different structure. For example, the compound may comprise one type of duocarmycin, but in addition

no other type of duocarmycin, no other type of drug moiety and no other type of functional agent. Or, in another example, the compound may comprise one type of maytansine, but in addition no other type of maytansine, no other type of drug moiety and no other type of functional agent. The one type of functional agent (in the first example the one type of duocarmycin; in the second example the one type of maytansine) may be present in the compound in one or more copies.

Embodiment 363: The compound according to any one of embodiments 1 to 362, wherein said compound is an ADC (antibody-drug conjugate).

Embodiment 364: The compound according to any one of embodiments 5 to 363, wherein the Ligand unit is an antibody component (which functions as targeting antibody of the ADC) and the Functional agent is a drug payload (e.g. a cytotoxic agent).

As used herein, an "antibody-drug conjugate" (abbreviated "ADC") is a molecule comprising an antibody as Ligand unit, a Linker unit, and a payload (either a drug moiety or a detectable agent) as Functional agent. The different components of the antibody-drug conjugate are covalently linked.

By binding to its antigen, the antibody component of the ADC serves as targeting component that can direct the ADC to its target site. For example, if the antigen of the antibody component is a tumor antigen, the ADC will e.g. be directed to tumor cells expressing this tumor antigen at their cell surface. Upon recruitment of the ADC to the target site, the payload can mediate a therapeutic action (e.g. killing of a cancer cell, local reduction of an inflammation, local stimulation or suppression of the immune system).

Non-targeted drugs typically reach their site of action by whole-body distribution and passive diffusion. In contrast, ADCs are targeted compounds that are not distributed evenly across the whole body. Due to the interaction of the antibody component with its target antigen, an ADC is concentrated preferentially at its site target site. Therefore, ADCs with a therapeutic agent as payload require lower dosages to be therapeutically effective, thus improving the therapeutic window.

In many cases, upon binding to its target cell an ADC will be internalized into the cell, e.g. by receptor-mediated endocytosis. The Linker unit may be cleaved after cellular incorporation by enzymatic cleavage. Alternatively, the antibody may be degraded inside of the cell. In either case, the payload is released into the cellular interior. If the payload is a medical drug, it can then fulfill its therapeutic function inside of the cell. If the payload is a detectable agent the agent may be detected e.g. to identify the target site.

Antibody-drug conjugates, their structure, preparation and use are described in detail e.g. in Antibody-Drug Conjugates: Fundamentals, Drug Development, and Clinical Outcomes to Target Cancer, 1st edition (2016), editors Olivier and Hurvitz, publisher John Wiley & Sons, Inc. (U.S.); Toader, Topics in Medicinal Chemistry (2018), vol. 28 (Cancer II), p. 289-332; Chau, Lancet (2019), vol. 394 (10200), p. 793-804; Nimoy, Pharmaceuticals (2018), vol. 11 (2), p. 32/1-32/22; Fei et al., Journal of Biomedical Nanotechnology (2018), vol. 14(3), p. 405-429; Gorka et al., Accounts of Chemical Research (2018), vol. 51(12), p. 3226-3235; Tiberghien et al., Journal of Organic Chemistry (2019), vol. 84(8), p. 4830-4836; Rohrer, in: Process Scale Purification of Antibodies, 2nd edition (2017), editor: Gottschalk, John Wiley & Sons, Inc., p. 595-614; Vaklavas and Forero, Methods in Molecular Biology (2012), vol. 899, p. 489-497.

The term "payload", as used herein, refers to a chemical moiety that is conjugated to an antibody component as part of an antibody-drug conjugate. In the antibody-drug conjugate according to the present disclosure, the payload is linked to the antibody component by covalent binding through a linker. As described above, the payload in the ADC of the present disclosure (resp. the functional moiety) is a therapeutic agent or a detectable label. Upon recruitment of the ADC to its target site by binding of the antibody component to its target antigen, the payload can fulfill its function at the target site. For example, if the antibody component is specific for the tumor antigen, the payload may be a cytotoxic agent that kills tumor cells, e.g. a maytansinoid or duocarmycin. Or if the antibody component is specific for an antigen indicating inflammation, the payload may be an anti-inflammatory agent, e.g. a glucocorticoid receptor antagonist like cortisol or prednisolone. Or the payload may be a detectable agent that allows to detect the presence of the target antigen or identify the target site.

The payload can be introduced into the ADC at different stages of preparation. In one approach, a linker-payload construct (i.e. a construct in which the payload is covalently linked to the

linker) is synthesized by standard methods of organic chemistry (as shown in the examples) and subsequently this linker-payload payload construct is conjugated to the antibody component. However, the antibody component, linker and payload can also be prepared and conjugated in different order (e.g. the linker is conjugated to the antibody component and subsequently the payload attached to the linker).

Different payloads, their preparation, conjugation and use in antibody-drug conjugates are described e.g. in Nicolaou et al., Accounts of Chemical Research (2019), vol. 52(1), p. 127-139; Maderna et al., Molecular Pharmaceutics (2015), vol. 12(6), p. 1798-1812; Gromek et al., Current Topics in Medicinal Chemistry (2014), vol. 14(24), p. 2822-2834.

The ADC according to the present disclosure may comprise only one type of payload (i.e. one ADC molecule is linked to only one kind of payload, e.g. auristatin E, wherein one or more copies of the payload (in this example auristatin E) may be linked to the ADC molecule) or several types of payloads (i.e. one ADC molecule is linked to two or more kinds of payload, e.g. auristatin E and DM4, wherein one or more copies of each payload (in this example one or more copies of auristatin E and one or more copies of DM4) may be linked to the ADC molecule). Preferably, the antibody-drug conjugate according to the present disclosure comprises only one kind of payload.

The copy number payloads linked to one ADC molecule (i.e. in the first example above the number of auristatin E molecules linked to one ADC molecule, and in the second example above the number of auristatin E molecules plus the number of DM4 molecules linked to one ADC molecule) is reflected in the drug-antibody ratio.

As a skilled person will understand, in practice ADCs are often populations of molecules that slightly vary with regard to their characteristics. For example, a population of ADC molecules may for the most part include ADC molecules with 4 payloads per ADC molecule, but may also contain a small fraction of ADC molecules with 3 payloads and a small fraction of ADC molecules with 5 payloads per ADC molecule. In such a case where there is slight variation in an ADC population with regard to a characteristic, the numbers indicated below will typically relate to the rounded average number over the population.

For the purposes of the present disclosure, a high homogeneity between the ADCs within the population of interest is usually desirable. A higher homogeneity can typically be achieved by additional steps of purification/separation, e.g. by HIC (hydrophobic interaction chromatography), SEC (size exclusion chromatography) and HPLC/reversed phase HPLC. The homogeneity of an ADC population can be determined e.g. by HIC, HPLC/reversed phase HPLC, SDS-PAGE analysis and MS (mass spectrometry) analysis. For an analysis differentiating between the antibody heavy and light chain, SEC under reducing conditions or SDS-PAGE followed by MS analysis can be carried out.

The copy number payloads linked to one ADC molecule (i.e. in the first example above the number of auristatin E molecules linked to one ADC molecule, and in the second example above the number of auristatin E molecules plus the number of DM4 molecules linked to one ADC molecule) is reflected in the drug-antibody ratio.

As used herein, the "drug-antibody ratio" of an ADC (abbreviated as "DAR") is the (average) number of payloads (or generally, Functional agents) per ADC molecule divided by the number of antibody components per ADC molecule. The DAR of an ADC can e.g. be determined by identifying the molecular components of an ADC molecule by mass spectrometry and subsequently dividing the number of Functional agents (e.g. drug moyeties or detectable labels, if the Functional agent is a detectable label) in an ADC molecule to the number of antibody components in the ADC molecule (the ADC according to the present disclosure contains one antibody component per ADC molecule). The DAR values of the embodiments defined below are preferably determined by this approach, i.e. calculated from structural information obtained by mass spectrometry.

ADCs with different DAR can be prepared by linking different numbers of payloads to the ADC molecule. For example, a linker-payload construct including one payload copy per linker can be prepared, and subsequently multiple copies of this linker-payload construct are linked to each antibody component. The number of linker-payload constructs that are linked per antibody component can be influenced by the reaction conditions (e.g. the concentrations of the components, degree of activation of components, duration of conjugation reaction etc.), as known to a skilled person and described in Example 3 below. See also section on Conjugation below.

Embodiment 365: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 1 to 20.

Embodiment 366: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 1 to 10.

Embodiment 367: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 1 to 8.

Embodiment 368: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 1 to 4.

Embodiment 369: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 1 to 2.

Embodiment 370: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 2 to 6.

Embodiment 371: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 4 to 6.

Embodiment 372: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 2 to 8.

Embodiment 373: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 4 to 8.

Embodiment 374: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 6 to 8.

Embodiment 375: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 2 to 10.

Embodiment 376: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 4 to 10.

Embodiment 377: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 6 to 10.

Embodiment 378: The compound according to any one of embodiments 1 to 364, wherein said compound has a DAR in the range of from 8 to 10.

Embodiment 379: The compound according to any one of embodiments 1 to 378, wherein said self-immolative group/said self-immolative spacer undergoes, upon cleavage of the chemical bond that triggers self-immolation, intramolecular cleavage with a half-life of below 2 hours in water at 37° C at a pH of 7.4.

Embodiment 380: The compound according to any one of embodiments 1 to 379, wherein said compound is a molecule.

Embodiment 381: The compound according to any one of embodiments 1 to 380, wherein said compound comprises a protein.

By stating that the compound "comprises" a protein, the present disclosure designates that the compound includes a part within its chemical structure that is a protein. As the skilled person understands from the formulas provided, a compound according to the present disclosure includes within its chemical structure (also) a part that is not a protein.

Embodiment 382: The compound according to any one of embodiments 1 to 380, wherein said compound is a small molecule compound.

Embodiment 383: The compound according to any one of embodiments 1 to 381, wherein said compound comprises a protein and a small molecule covalently linked thereto.

Embodiment 384: The compound according to any one of embodiments 1 to 383, wherein all components of said compound are covalently linked (except for non-covalent association of

protein chains). Such non-covalent association of protein chains may for example occur within the Ligand unit, if the Ligand unit is formed by an antibody.

Embodiment 385: The compound according to any one of embodiments 1 to 384, wherein all components of said compound are covalently linked.

Embodiment 386: The compound according to any one of embodiments 1 to 385, wherein all units of said compound are covalently linked.

As the skilled person understands, the lines connecting different molecular components in the formula depictions herein indicate covalent bonds.

Embodiment 387: The compound according to any one of embodiments 1 to 386, wherein said Ligand unit, said Stretcher unit, said Iduronide unit, said Spacer unit and said Functional agent are covalently linked.

Embodiment 388: The compound according to any one of embodiments 1 to 387, wherein said Ligand unit, said optional Stretcher unit, said Iduronide unit, said optional Spacer unit and said Functional agent are covalently linked.

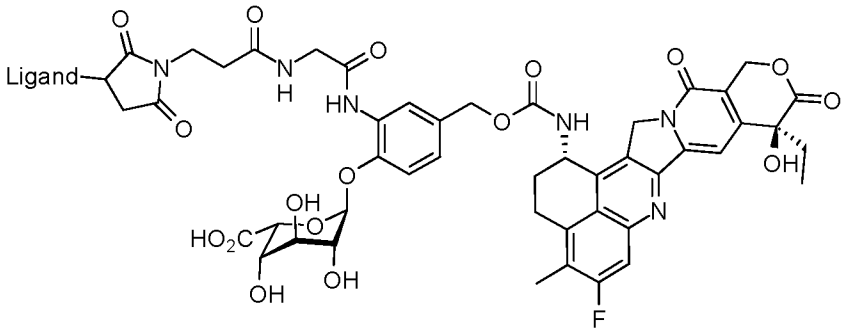
Embodiment 389: The compound according to any one of embodiments 1 to 388, wherein said Ligand unit, said Iduronide unit, said optional Spacer unit and said Functional agent are covalently linked.

Embodiment 390: The compound according to any one of embodiments 1 to 389, wherein said Ligand unit, said optional Stretcher unit, said Iduronide unit and said Functional agent are covalently linked.

Embodiment 391: The compound according to any one of embodiments 1 to 390, wherein said Ligand unit, said Iduronide unit, and said Functional agent are directly or indirectly covalently linked. "Directly" covalently linked means that there is only a covalent bond linking said units. "Indirectly" covalently linked means that said units are linked via another chemical group.

Embodiment 392: The compound according to any one of embodiments 1 to 391, wherein said Ligand unit, said Iduronide unit, and said Functional agent are covalently linked.

Embodiment 393: The compound according to any one of embodiments 1 to 392, wherein said compound has a structure according to the following formula



(Formula 53).

Figure 13 shows how the Stretcher unit, Iduronide unit, Spacer unit, self-immolative group and Functional agent can be reflected in the chemical structure of a compound according to Formula 53.

Embodiment 394: The compound according to any one of embodiments 1 to 393, wherein *a* is 0 or 1.

Embodiment 395: The compound according to any one of embodiments 1 to 393, wherein *a* is 1.

Embodiment 396: The compound according to any one of embodiments 1 to 393, wherein *a* is 0.

Embodiment 397: The compound according to any one of embodiments 1 to 396, wherein *w* is 1.

Embodiment 398: The compound according to any one of embodiments 1 to 396, wherein *y* is 0 or 1.

Embodiment 399: The compound according to any one of embodiments 1 to 398, wherein *y* is 1.

Embodiment 400: The compound according to any one of embodiments 1 to 398, wherein *y* is 0.

Embodiment 401: The compound according to any one of embodiments 1 to 393, wherein

a is 0 or 1;

w is 1; and

y is 0, 1 or 2.

Embodiment 402: The compound according to any one of embodiments 1 to 393, wherein

a is 0 or 1;

w is 1; and

y is 0 or 1.

Embodiment 403: The compound according to any one of embodiments 1 to 402, wherein *f* is 1 or 2.

Embodiment 404: The compound according to any one of embodiments 1 to 402, wherein *f* is 2.

Embodiment 405: The compound according to any one of embodiments 1 to 402, wherein *f* is 1.

Embodiment 406: The compound according to any one of embodiments 1 to 402, wherein *f* is 2 to 4.

Embodiment 407: The compound according to any one of embodiments 1 to 402, wherein *f* is 3 or 4.

Embodiment 408: The compound according to any one of embodiments 1 to 407, wherein *p* is 1 to 20.

Embodiment 409: The compound according to any one of embodiments 1 to 407, wherein *p* is 1 to 10.

Embodiment 410: The compound according to any one of embodiments 1 to 407, wherein *p* is 1 to 8.

Embodiment 411: The compound according to any one of embodiments 1 to 407, wherein *p* is from 1 to 4.

Embodiment 412: The compound according to any one of embodiments 1 to 407, wherein *p* is from 1 to 2.

Embodiment 413: The compound according to any one of embodiments 1 to 407, wherein *p* is from 2 to 6.

Embodiment 414: The compound according to any one of embodiments 1 to 407, wherein *p* is from 4 to 6.

Embodiment 415: The compound according to any one of embodiments 1 to 407, wherein *p* is from 2 to 8.

Embodiment 416: The compound according to any one of embodiments 1 to 407, wherein *p* is from 4 to 8.

Embodiment 417: The compound according to any one of embodiments 1 to 407, wherein *p* is from 6 to 8.

Embodiment 418: The compound according to any one of embodiments 1 to 407, wherein *p* is from 2 to 10.

Embodiment 419: The compound according to any one of embodiments 1 to 407, wherein *p* is from 4 to 10.

Embodiment 420: The compound according to any one of embodiments 1 to 407, wherein *p* is from 6 to 10.

Embodiment 421: The compound according to any one of embodiments 1 to 407, wherein *p* is from 8 to 10.

As a skilled person understands, at the level of an individual compound, p is an integer number.

Embodiment 422: The compound according to any one of embodiments 1 to 421, wherein *p* is an integer number.

If considered over a whole population, *p* is an average number which is determined over a population of said compound molecules. Thus, in this case also numbers between integer numbers can occur. Accordingly, in some embodiments, p is a floating point number. Preferably, said population is a homogeneous population.

Fifth aspect of the present disclosure (also referred to as "Embodiment 423"): According to a fifth aspect, the present disclosure relates to a method for preparing a compound according to any one of embodiments 5 to 422, wherein said method comprises the step of covalently linking at least one molecule comprising a Functional agent to a molecule comprising a Ligand unit.

Sixth aspect of the present disclosure (also referred to as "Embodiment 424"): According to a sixth aspect, the present disclosure relates to a molecule for use in the preparation of a compound according to any one of embodiments 5 to 422, wherein said molecule comprises an Iduronide unit that is covalently linked to an activator group.

Seventh aspect of the present disclosure (also referred to as "Embodiment 425"): According to a seventh aspect, the present disclosure relates to the use of a molecule in the preparation of a compound according to any one of embodiments 5 to 422, wherein said molecule comprises an Iduronide unit covalently linked to an activator group.

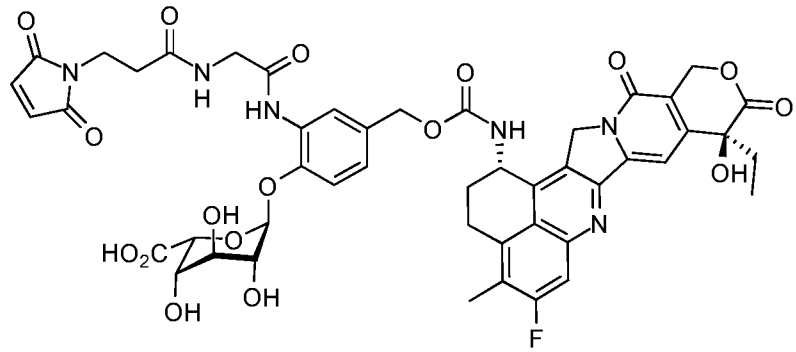
Preparation of a compound according to the present disclosure may be achieved by "activating" the Linker unit or the Functional agent (i.e. by forming an intermediate with a reactive chemical

group) and subsequently carrying out a reaction in which that activated intermediate is covalently linked to the other components of the compound according to the present disclosure.

As used herein, an "activator group" is a reactive chemical group useful for covalently linking the Iduronide unit to another molecule, e.g. a Ligand unit (such as an antibody) or a Functional agent (e.g. a drug payload of an ADC), as defined in the present disclosure. Thus, the above-described molecule comprising an Iduronide unit covalently linked to an activator group can be used advantageously as a "building block" for the preparation of a compound according to the present disclosure by standard methods of synthetic organic chemistry. As the skilled person understands, the reactive groups must be selected based on compatibility and selectivity as described above for conjugation reactions.

Said activator group may be an activator group for chemical coupling, such as a maleimide, an *N*-hydroxysuccinimide ester, a halogen-acetamide, an alkyl halogen, a Michael acceptor (wherein said Michael acceptor is preferably a vinyl-pyridine) and a group suitable for cycloaddition (wherein said group suitable for cycloaddition is preferably a ketone, hydrazone, semicarbazone, carboxylic acid, alkene or alkyne suitable for cycloaddition). Alternatively, the activator group may be an activator group for enzymatic coupling, such as a triple glycine GlyGlyGly (for sortase oder transglutaminase coupling) or a primary amine (for transglutaminase coupling).

For example, if the compound according to the present disclosure is an ADC composed of an antibody as ligand, a Stretcher unit, an Iduronide unit, a Spacer unit and exatecan as Functional agent, a molecule as shown below can be used for preparation of said compound:



(Formula 54)

This molecule is a drug-linker including a maleimide group as activator group. To attach this activated drug-linker to a ligand, the activated drug-linker may e.g. be conjugated via the maleimide to the interchain cystein of an antibody that serves as Ligand unit.

Embodiment 426: The use according to any one of embodiments 424 to 425, wherein said activator group is an activator group for chemical coupling.

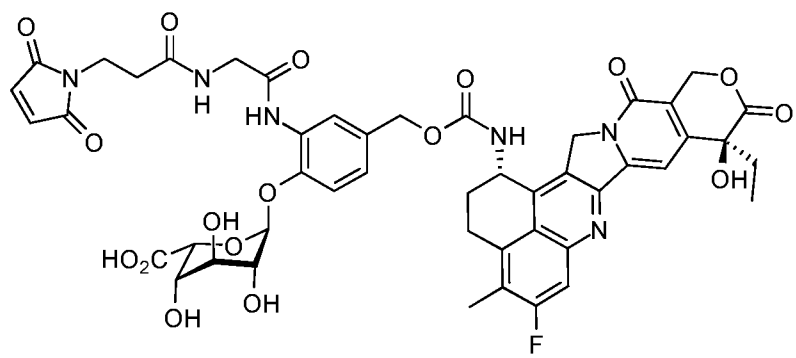
Embodiment 427: The use according to any one of embodiments 424 to 426, wherein said activator group is selected from the group consisting of a maleimide, an *N*-hydroxysuccinimide ester, a halogen-acetamide, an alkyl halogen, a Michael acceptor (wherein said Michael acceptor is preferably a vinyl-pyridine) and a group suitable for cycloaddition (wherein said group suitable for cycloaddition is preferably a ketone, hydrazone, semicarbazone, carboxylic acid, alkene or alkyne suitable for cycloaddition).

Embodiment 428: The use according to any one of embodiments 424 to 427, wherein said activator group is an activator group for enzymatic coupling.

Embodiment 429: The use according to any one of embodiments 424 to 428, wherein said activator group is a triple glycine GlyGlyGly or a primary amine.

Embodiment 430: The use according to any one of embodiments 424 to 429, wherein said activator group is a maleimide.

Embodiment 431: The use according to any one of embodiments 424 to 430, wherein said molecule has a structure according to the following formula:



(Formula 54).

Eighth aspect of the present disclosure (also referred to as "Embodiment 432"): According to an eighth aspect, the present disclosure relates to a method for increasing the cytotoxicity of a molecule, said molecule comprising a Ligand unit, wherein said method comprises covalently linking at least one Functional agent to said molecule, thus providing a compound according to any one of embodiments 5 to 422, wherein said Functional agent is a cytotoxic agent (i.e. a therapeutic agent which is a cytotoxic agent).

If the present disclosure indicates that a modification or action is for "increasing the cytotoxicity of a molecule ", this refers to a situation where the cytotoxicity of said molecule after carrying out said method is higher than before carrying out said method.

Preparation of a "compound in which said molecule is covalently linked to at least one Functional agent" may be achieved either by preparing and characterizing the individual components (i.e. the Ligand unit, Linker unit and Functional agent) as described above for the method for increasing the solubility of a molecule, and then linking them covalently. The order in which the individual components are linked is not limited. Thus, it is e.g. possible to prepare by methods of synthetic organic chemistry a Linker unit covalently linked to a Functional agent and conjugate this construct in a final step to the Ligand unit by standard methods of conjugation as described in the literature and in Example 1 below). Or it is possible to first prepare a Linker unit covalently linked to a Ligand unit and subsequently carry out a reaction for covalent attachment of a cytotoxic agent as Functional agent to the Ligand unit-Linker unit construct.

For covalent linkage of the individual components, any standard methods of synthetic organic chemistry and enzymatic reactions can be used (e.g. Behrens et al., Molecular Pharmaceutics (2015), vol. 12(11), p. 3986-3998; Stefano, Methods in Molecular Biology (2013), vol. 1045, p. 145-171; Dickgiesser et al., in: Methods in Molecular Biology: Enzyme-Mediated Ligation Methods (2019), editors Nuijens and Schmidt, vol. 2012, p. 135–149; Dickgiesser et al., Bioconjugate Chem. (2020), vol. 31(4), p. 1070-1076)), provided that the chosen methods do not damage the molecular components to be linked. Confirmation of successful preparation of the compound according to the present disclosure including a cytotoxic agent and verification

that inclusion of the Functional agent indeed results in an increased cytotoxicity can then be carried out as described above and in the examples.

An increase of the cytotoxicity of a molecule A comprising a Ligand unit by covalent attachment of a molecule B can for example be determined in a tissue culture assay. Target cells to which said Ligand unit binds are cultured by ways of tissue culture. To the target cells, either said molecule A with molecule B covalently attached or said molecule A without molecule B covalently attached is added. Cytotoxicity can be determined by subsequently determining the microscope the number of target cells killed upon addition of said molecule A with resp. without molecule B covalently attached to it. If desired, non-target cells (i.e. cells to which said Ligand unit does not bind to) can be included in the experiment to determine the specificity of the observed effects.

Nineth aspect of the present disclosure (also referred to as "Embodiment 433"): According to a nineth aspect, the present disclosure relates to a method for increasing the cytotoxicity of a molecule, said molecule comprising a Ligand unit, but no Functional agent, wherein said method comprises the preparation of a compound according to any one of embodiments 5 to 422, wherein said Functional agent is a cytotoxic agent (i.e. a therapeutic agent which is a cytotoxic agent).

Tenth aspect of the present disclosure (also referred to as "Embodiment 434"): According to a tenth aspect, the present disclosure relates to the use of a molecule comprising a Functional agent for increasing the cytotoxicity of a molecule comprising a Ligand unit, wherein said use involves the step of covalently linking at least one molecule comprising a Functional agent to said molecule comprising a Ligand unit, thus providing a compound according to any one of embodiments 5 to 422, wherein said Functional agent is a cytotoxic agent (i.e. a therapeutic agent which is a cytotoxic agent).

The definitions and explanations provided below relate to any of the aspects, compounds, methods or uses defined above or below in this disclosure.

Eleventh aspect of the present disclosure (also referred to as "Embodiment 435"): According to an eleventh aspect, the present disclosure relates to a pharmaceutical composition comprising the compound according to any one of embodiments 5 to 422.

Methods for preparing pharmaceutical compositions are known to a skilled person in the art (Remington: The Science and Practice of Pharmacy, 22nd ed. (2012), Pharmaceutical Press).

Embodiment 436: The pharmaceutical composition according to embodiment 435, wherein said pharmaceutical composition comprises a pharmaceutically acceptable carrier, diluent and/or excipient.

The term "pharmaceutically acceptable" designates that said carrier, diluent or excipient is a non-toxic, inert material that is compatible with the other ingredients of the pharmaceutical composition and not harmful to the patient that the pharmaceutical composition is administered to, such that it can be used in a pharmaceutical product. Substances suitable as carriers, diluents or excipients in pharmaceutical compositions are known to a skilled person in the art (Remington: The Science and Practice of Pharmacy, 22nd ed. (2012), Pharmaceutical Press). The pharmaceutical composition may further include e.g. additional adjuvants, antioxidants, buffering agents, bulking agents, colorants, emulsifiers, fillers, flavoring agents, preservatives, stabilizers, suspending agents and/or other customary pharmaceutical auxiliaries.

Embodiment 437: The pharmaceutical composition according to any one of embodiments 435 to 436, wherein said pharmaceutical composition further includes at least one additional adjuvant, antioxidant, buffering agent, bulking agent, colorant, emulsifier, filler, flavoring agent, preservative, stabilizer, suspending agent and/or other customary pharmaceutical auxiliary.

Embodiment 438: The compound according to any one of embodiments 5 to 422 or the pharmaceutical composition according to any one of embodiments 435 to 437 for use as a medicament.

Embodiment 439: The compound according to any one of embodiments 5 to 422 or 438 or the pharmaceutical composition according to any one of embodiments 435 to 437 for use in the treatment of a disease.

Embodiment 440: The compound according to any one of embodiments 5 to 422 or 438 to 439 or the pharmaceutical composition according to any one of embodiments 435 to 437 for use in the treatment of cancer.

Embodiment 441: The compound according to any one of embodiments 5 to 422 or 438 to 440 or the pharmaceutical composition according to any one of embodiments 435 to 437 for use in the treatment of a solid malignant tumor.

Embodiment 442: The compound according to any one of embodiments 5 to 422 or 438 to 441 or the pharmaceutical composition according to any one of embodiments 435 to 437 or the pharmaceutical composition for use according to any one of embodiments 438 to 441, wherein said compound/said pharmaceutical composition is for use in the treatment of a human.

The production of medicaments containing the compound of the present disclosure according or a pharmaceutical composition according to the present disclosure can be performed according to well-known pharmaceutical methods. Further details on techniques for formulation and administration may be found e.g. in Remington: The Science and Practice of Pharmacy, 22nd ed. (2012), Pharmaceutical Press.

As used herein, "treatment" of a disease and "treating" a disease refers to the process of providing a subject with a pharmaceutical treatment, e.g., the administration of a drug, such that said disease is alleviated, reduced, minimized, halted or even healed, and/or such that the chances of a relapse into the disease are reduced or a relapse into the disease is even prevented.

The use of compounds in the treatment of diseases is known to a skilled person in the art (see e.g. Coats et al., Clinical Cancer Research (2019), vol. 25(18), p. 5441-5448; Rudra, Bioconjugate Chemistry (2020), vol. 31(3), p. 462-473). Thus, the skilled person is aware that the components of the compound, in particular the targeting moiety, must be selected appropriately in order to allow for successful treatment. For example, for treatment of a specific cancer, the Ligand unit of the compound (for example an antibody or antigen-binding fragment thereof) must be selected such that binding of the Ligand unit to its target site directs the compound to said cancer (e.g. by using as Ligand unit an antibody against a tumor-associated antigen that is specifically found on the surface of the cancer cells). The Functional agent of said compound can then be selected such that a therapeutic effect is achieved. For example, the

Functional agent can be a cytotoxic agent that kills the cancer cells to which the compound is recruited.

Twelfth aspect of the present disclosure (also referred to as "Embodiment 443"): According to a twelfth aspect, the present disclosure relates to a method for treating a disease in a patient in need thereof, comprising the step of administering to said patient a therapeutically effective amount of the compound according to any one of embodiments 5 to 422 or the pharmaceutical composition according to any one of embodiments 435 to 437.

By "therapeutically effective amount" is meant the amount of an agent required to ameliorate the symptoms of a disease. The effective amount of active agent(s) (e.g., a compound according to the present disclosure) used for therapeutic treatment of a disease according to the present disclosure varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as a "therapeutically effective" amount.

The term "patient", as used herein, refers to a mammal (such as a human, rat, mouse, monkey, pig, goat, cow, horse, dog or cat). Preferably, the patient is a human.

Embodiment 444: The method according to embodiment 443, wherein said disease is cancer.

As used herein, the term "cancer" refers to a malignant neoplasm. Cancer can include a hematological cancer or a solid tumor.

Embodiment 445: The method according to any one of embodiments 443 to 444, wherein said disease is a malignant tumor.

Embodiment 446: The method according to any one of embodiments 443 to 445, wherein said disease is a solid malignant tumor.

Embodiment 447: The method according to any one of embodiments 443 to 446, wherein said patient is a human.

Thirteenth aspect of the present disclosure (also referred to as "Embodiment 448"): According to a thirteenth aspect, the present disclosure relates to the use of the compound according to any one of embodiments 5 to 422 or the pharmaceutical composition according to any one of embodiments 435 to 437 for the manufacture of a medicament.

Fourteenth aspect of the present disclosure (also referred to as "Embodiment 449"): According to a fourteenth aspect, the present disclosure relates to the use of the compound according to any one of embodiments 5 to 422 or the pharmaceutical composition according to any one of embodiments 435 to 437 for the manufacture of a medicament for the treatment of cancer.

Fifteenth aspect of the present disclosure (also referred to as "Embodiment 450"): According to a fifteenth aspect, the present disclosure relates to the use of the compound according to any one of embodiments 5 to 422 or the pharmaceutical composition according to any one of embodiments 435 to 437 for the manufacture of a medicament for the treatment of a malignant tumor.

Embodiment 451: The use according to embodiment 450, wherein said medicament is prepared for administration to a human.

Embodiment 452: The compound or the pharmaceutical composition for use according to any one of embodiments 438 to 442 or the method according to any one of embodiments 443 to 447 or the use according to any one of embodiments 448 to 451, wherein said cancer or malignant tumor is a human disease.

Embodiment 453: The compound according to any one of embodiments 1 to 422 or the pharmaceutical composition according to any one of embodiments 435 to 437 or the compound or pharmaceutical composition for use according to any one of embodiments 438 to 442 or 452 or the method according to any one of embodiments 443 to 447 or 452 or the use according to any one of embodiments 448 to 452, wherein

Ligand is a Ligand unit;

A is a Stretcher unit;

a is 0, 1 or 2;

W is an Iduronide unit;

w is 1 or 2;

Y is a Spacer unit;

y is 0, 1 or 2;

A_a–W_w–Y_y is a Linker unit;

F is a Functional agent;

f is 1, 2, 3 or 4;

A_a–W_w–Y_y–F_f is a Linker-Functional agent unit;

p is from 1 to 20.

Sixteenth aspect of the present disclosure (also referred to as "Embodiment 454"): According to a sixteenth aspect, the present disclosure relates to a method of reducing cellular proliferation, the method comprising contacting a cell with an anti-proliferative amount of a compound according to any one of embodiments 1 to 422 or 453 or with an anti-proliferative amount of a pharmaceutical composition according to any one of embodiments 435 to 437 or 453.

EXAMPLES

The following examples describe the preparation and characterization of iduronidase-cleavable linkers as disclosed in the present disclosure, as well as related compounds (such as ADCs) and methods, along with comparative disclosure. It is understood that various embodiments of the disclosure reflected in the examples may be practiced, given the general description provided above. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the description and examples should not be construed as limiting the scope of the invention.

Example 1

Preparation of antibodies

The antibody preparation was conducted as previously described (Jaeger et al., 2021). In brief, anti-Ceacam5 mAb was expressed by a transient transfection of heavy chains and light chains in Expi293F cells following the manufacturer's instructions using the corresponding transfection kit and media from Life Technologies. Supernatant was harvested 5 days post transfection. Antibodies were purified by protein A affinity chromatography using HiTrap Mab Select SuRe 5 mL columns (GE Healthcare) and subsequently formulated in PBS at pH 6.8

using HiPrep 26/10 desalting columns using an ÄKTA Pure device (GE Healthcare). Antibody purity was analyzed by analytical SE-HPLC using a TSKgel SuperSW3000 column (Tosoh Bioscience) and by SDS gel electrophoresis. The identity of proteins was confirmed via an intact mass analysis by LC-MS using a TripleTOF 6600+ mass spectrometer (AB Sciex). Antibodies were concentrated using Ultra centrifugal filter units (3000 molecular weight cutoff (MWCO), Amicon) and sterile filtered, and the protein concentration was determined by UV−vis spectroscopy at 280 nm. Antibody fragments were snap-frozen in liquid nitrogen and stored at −80 °C.

Cell lysate generation

For the generation of cell lysates, cells (mononuclear cells (MNC, Lonza), peripheral blood mononuclear cells (PBMC, Charles River), normal human dendritic cells (NHDC, Lonza), normal human epidermal keratinocytes (NHEK, Promo Cell), human umbilical vein (HUVEC, Lonza)) were harvested at 2000 rpm for 2 min, followed by application of three wash cycles with PBS. After final suspension of cells in 300 µL cell lysis buffer (100mM Na-Citrate, 1mM Na3-EDTA, 1% Octyl-ß-glucopyranoside pH 5.8), three consecutive freeze-thaw cycles were applied at -80 °C followed by removal of cell debris by centrifugation at 10,000 rpm for 12 min and final storage at -80 °C.

In vitro drug release assay with cellular extracts

Maleimide payload-linker constructs were quenched with N-acetylcysteine to avoid adduct formation. For this, 100 mM N-acetylcysteine solution was prepared in DI-water with 0.1 M Bis-Tris buffer pH 7.0, 15% (v/v) DMSO and 100 µM linker-payload. Samples were supplemented with 100 µM deuterated 7-ethyl-d3-camptothecin (D3-CPT) as an internal standard and incubated at room temperature for 1 h. Afterwards, 5 µM quenched linker-payload and D3-CPT was incubated together with 20 µg cell lysate or lysosomal extract (Xenotech, H0610.L) in a total volume of 100 µL of catabolism buffer (Xenotech, pH 5.0) at 37 °C for up to 48 h. Time-series of 10 µL samples were taken, each mixed with 40 µl methanol supplemented with 1:200 Protease Inhibitor Cocktail Set III (Merck KGaA) for protein precipitation followed by centrifugation at 10,000 rpm for 5 min and quantification of Exatecan in the supernatant *via* LC-MS/MS.

In vitro drug release assay with recombinant enzymes

For cleavage assays, recombinant human α-L-Iduronidase (IDUA, R&D Systems) was incubated together with fluorescent substrates to demonstrate IDUA substrate specificity. The compound 4-methylumberlliferyl-α-L-iduronide (ChemCruz) served as substrate for IDUA. The compounds 4-Methylumbelliferyl-β-D-glucuronide (Sigma) and 4-Methylumbelliferyl-β-D-galactopyranoside (Sigma) were included as alternative substrates. In brief, IDUA and fluorescent substrates were separately diluted in respective assay buffers according to the manufacturer's instructions. Enzyme and substrate containing assay buffers were combined on F16 Black Maxisorp Plates (Thermofisher) and fluorescence was measured on a plate reader (Synergy Neo2, Biotek) for 6 h at excitation and emission wavelengths of 365 and 445 nm, respectively. Substrate blank measurements without enzymes were included to calculate the specific activity.

Besides fluorescence substrates, quenched linker-payloads were applied in cleavage assays with their specific enzymes (IDUA or recombinant human Cathepsin B (CatB, R&D Systems)). For IDUA cleavage, 5 µM quenched linker-payload was incubated with 0.25 ng/µL IDUA in 100 mM sodium acetate buffer at pH 4.5. For CatB cleavage, 0.25 ng/µL CatB was activated at room temperature with 2 mM dithiothreitol (DTT) in 100 mM sodium acetate, 1 mM EDTA buffer at pH 5.0 for 20 min. Afterwards, 5 µM quenched linker-payload was added. Enzymatic reaction mixes were incubated at 37 °C for 3 h and the reaction was analyzed by LC-MS every 12 min to follow enzymatic cleavage. Separated analytes were detected at 360 nm. Peak integration and quantification were performed with the SCIEX OS software. Peak areas were normalized to the total peak area of the t_0 sample and plotted as percentage of peak area. Resulting curves were fitted and the degradation constants were calculated using the following coupled reaction kinetics:

$$[Exatecan] = [LP]_0 * \left(1 - \frac{k_2}{k_2 - k_1} e^{-k_1 t} + \frac{k_1}{k_2 - k_1} e^{-k_2 t}\right)$$

ADC preparation

ADC preparation with constructs **12**, **13**, **14** was carried out as previously described (Jaeger et al., 2021; Kaempffe et al., 2021). Antibody was diluted to a final concentration of 5 mg/mL in 1 mM EDTA and partially reduced with an excess of 40 equiv of tris(2-carboxyethyl) phosphine (TCEP) for 2 h at 37 °C. TCEP was removed *via* two consecutive 5 mL HiTrap Desalting

Columns (GE Healthcare), and the reduced antibody was reoxidized with 20 equiv of dehydroascorbic acid for 2 h at 25 °C. To this mixture, 8 equiv of linker payload (1, 2 or 3) were added and incubated for 1 h at 25 °C before the reaction was stopped by the addition of 25 equiv of N-acetylcysteine (15 min at 25 °C) and purified by preparative SEC. Preparative SEC was performed using a HiLoad Superdex 200 prep grade 16/60 column in a 1260 liquid chromatography system (Agilent Technologies) with PBS at pH 6.8 as a running buffer. Purified ADCs were concentrated using centrifugal filter units (Amicon) and sterile-filtered. Protein concentration was determined by UV−vis spectroscopy at 280 nm and purified conjugates were subjected to analysis by SE-HPLC and DAR determination (HI-HPLC, RP-HPLC, LC-MS) as described elsewhere (Dickgiesser et al., 2020), snap-frozen in liquid nitrogen, and stored at −80 °C.

Conjugation of MC-iduronide-duocarmycin (**15**) was conducted as previously described in Dickgiesser et al., 2020. In brief, site-specific conjugation of a reactive handle cysteamine to native glycosylated antibody was performed using engineered MTG (microbial transglutaminase), followed by drug-linker attachment in a second step. For the preparative conjugation of reactive handle cysteamine to native antibody via MTG, 34.4 µM (1 eq.) antibody was mixed with 20 eq. cysteamine and 100 U/mL MTG in buffer with 10% DMSO. The solution was incubated for 40 h at 37 °C with gentle shaking, stopped with MTG blocker (Zedira) and subjected to SEC purification. Resulting cysteamine-conjugate solution (34.4 µM, 1 eq.) was mixed with 40 eq. TCEP in PBS pH 7.4 containing 1 mM EDTA and incubated for 2 h at 37 °C for full reduction. Afterwards, TCEP was removed via desalting with PD MiniTrap column according to the manufacturer's gravity protocol. The reaction mixture was concentrated using Amicon Ultra-2 concentrator units to a concentration >34.4 µM. Next, 20 eq. dhAA were added for re-oxidation and it was incubated for 2 h at 37 °C followed by the addition of 6 eq. of drug-linker (**15**) and incubation for 1 h at 25 °C. The reaction was quenched by addition of 25 eq. N-acetylcysteine and incubation for 0.5 h at 25 °C. The ADC was subsequently purified from the reaction mixture using SEC.

Serum stability

The serum stability assay was conducted as previously described (Dickgiesser et al., 2020; Jaeger et al., 2021; Kaempffe et al., 2021) applying some minor modifications: Exatecan conjugates were incubated at a final concentration of 5 µM conjugated exatecan (considering

the DAR (drug antibody ratio) of each construct) in human, mouse and cyno serum. Moreover, serum samples were supplemented with 5 µM deuterated 7-ethyl-d3-camptothecin (D3-CPT) as an internal standard.

Cancer cell cytotoxicity assay

Cell culture was conducted as previously described (Jaeger et al., 2021): Human cancer cell lines were obtained from the American Type Culture Collection (CEA5-positive: SK-CO-1, MKN-45, LS174T; CEA5-negative: MDA-MB-231) and maintained according to standard culture conditions (37 °C, 5% CO_2, 95% humidity). SK-CO-1 cells were cultured in Minimum Essential Medium Eagle (Sigma-Aldrich, FG0325) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1 mM sodium pyruvate. LS174T cells were cultured in Dulbecco′s Modified Eagle′s Medium (DMEM) (Sigma-Aldrich, FG0435) supplemented with 10% FBS, 1 mM sodium pyruvate and 1% (v/v) Non-Essential Amino Acid 100x mix (Sigma-Aldrich, M7145). MDA-MBA-231 cells were cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine and 1 mM sodium pyruvate. MKN-45 cells were cultured in Roswell Park Memorial Institute (RPMI) (Sigma-Aldrich, FG1215) 1640 medium supplemented with 10% FBS and 1 mM sodium pyruvate. For subculturing, adherent grown cells were detached by the addition of 0.05% trypsin−EDTA, diluted with fresh medium, and transferred into a new culturing flask. For the evaluation of exatecan and exatecan conjugates, 90 µL of viable cell (vc) suspension was seeded into 96-well plates (SK-CO-1 2,500 vc/well, MKN-45 500 vc/well, LS174T 2,500 vc/well, negative MDA-MB-231 1,500 vc/well) followed by incubation (37 °C, 5% CO_2) in a humid chamber overnight. Test compounds were formulated at 10-fold the starting concentration, prepared in serial dilution (1:4) and 10 µL were added to cells for each dilution in triplicates. The cell viability was determined after six days using Cell Titer Glo reagent (Promega) according to the manufacturer's instructions. Luminescence values were measured using a Varioskan plate reader (Thermo Fisher) and normalized to the luminescence of nontreated cells. Dose response was fitted using the using log (inhibitor) vs. response variable slope 4-parameter fitting function of GraphPad Prism (GraphPad Software, Inc.). Data was depicted as % relative cell viability versus molar compound concentration. Error bars indicating standard deviation (SD) of triplicates. Geometric mean values of IC50s derived from multiple experiments were calculated.

In vitro liver toxicity cell assay

Primary Human Liver Sinusoidal Microvascular Endothelial Cells (LSECs; Cell system, ACBRI 566 V, Lot: 566.04.03.05.0M) and Primary Human Hepatocytes (PHH; Gibco, HMCPIS, Lot: HU1951) were cultured according to the manufacturer's protocol. William's E medium without phenol red (Gibco, A1217601) containing 1x Penicillin-Streptomycin (Sigma-Aldrich, P4333), 1x GlutaMax (Gibco, 3505-061), 1x ITS (Sigma-Aldrich, I3146), dexamethasone, ascorbic acid and 1% (v/v) (LSECs) or 5% (v/v) (PHH) Fetal Bovine Serum (Sigma-Aldrich, F2442) was used for plating. For co-culture, plating medium was supplemented with 2.3% (v/v) Matrigel. For LSEC monoculture, 10,000 cells/well (100 µL) were seeded into a 96 well plate (Nunc Maxisorp). For LSEC/PHH co-culture, 10,000 LSEC cells/well and 50,000 PHH cells/well (100 µL) were seeded into a 96 well plate. Cells were treated with ADCs (900 nM), exatecan (250 nM), metformin (500 µM) as negative control, and tamoxifen (1, 20, 60 µM) and staurosporine (0.001, 0.1, 5 µM) as positive controls. Cells were incubated at 37 °C, 5% CO_2 for 24 h or 6 days (including medium exchange after 3 days), before ATP was measured using CellTiter-Glo assay (Promega, G7573) according to the manufacturer's protocol. Cell viability (%) was calculated based on the ATP luminescence signal and normalized to untreated cells (100%).

In vitro endothelial cell toxicity assay

Human Umbilical Vein Endothelial Cells (HUVEC; PromoCell, C-12203, Lot: 8071401) were cultured according to the manufacturer's protocol. Cells were diluted in Endothelial Cell Growth Medium MV (PromoCell, C-22020) and seeded into a 96 well plate (1000 cells/well, 100 µL/well). Cells were treated with ADCs (25 nM) or free exatecan (25 nM) at 37 °C, 5% CO_2 for 6 days before cell viability was analyzed using CellTiter-Glo assay (Promega, G7573) as described before.

In vivo xenograft study

Anti-tumor efficacy *in vivo* has been evaluated in the human gastric carcinoma xenograft model SNU-16. Six to eight week old immunodeficient female mice (C;129P2-H2d-TgH(Il2rg)tm1Bm-TgH(Rag2)tm1AltN4) were injected subcutaneously in the right flank with 5 million SNU-16 cells. When tumors reached a mean volume of 139 mm^3 (in the range of 105-201 mm^3, n=10 per treatment arm) mice were treated once intravenously with vehicle (saline

solution; 5 ml/kg) or with aCEA-duocarmycin ADC (5 mg/kg; 5ml/kg). Tumor size and body weight were examined twice per week. Tumor length (L) and width (W) were measured with calipers and tumor volumes were calculated using L×W^2/2.

Insofar as not noted otherwise, all methods in Examples 2 to 13 were carried out as described in Example 1.

Example 2

Iduronidase substrate cleavage and reduced normal tissue RNA expression

To evaluate the specificity of α-L-iduronidase substrate cleavage, recombinant α-L-iduronidase was co-incubated with fluorogenic substrates for α-L-iduronidase, β-D-glucuronidase, β-galactosidase. Substrate cleavage was measured by changes of relative fluorescence units over time (Figure 1 A). Interestingly, the iduronidase substrate 4-methylumbelliferyl α-L-iduronide (4-MU) was cleaved only by recombinant α-L-iduronidase, but did not serve as a substrate for β-D-glucuronidase and β-galactosidase, indicating great selectivity of IDUA substrate and enzyme.

Cathepsin B is a prominent lysosomal protease known to catalyze drug release from approved ADCs, but is also known to be widely and highly expressed in both diseased tissues and normal healthy cells. mRNA expression profiles of both α-L-iduronidase and cathepsin B were evaluated in normal healthy cells and several tumor tissues (Figure 1 B-D). To this end, α-L-iduronidase mRNA expression was evaluated based on data from the GTEx database encompassing endothelial cells of different normal tissues, human umbilical vein endothelial cells (HUVEC) and immune cells, representing the cell types most frequently affected by unspecific ADC uptake. The α-L-iduronidase mRNA expression was then compared to cathepsin B mRNA profiles (Figure 1 B and C). Surprisingly, α-L-iduronidase mRNA expression was much lower than cathepsin B mRNA expression profiles in all tissue and cell types.

In a next step, α-L-iduronidase mRNA expression was determined also in primary human tumors (stomach adenocarcinoma (STAD), colon adenocarcinoma (COAD), pancreatic adenocarcinoma (PAAD), liver hepatocellular carcinoma (LIHC)) and compared with normal adjacent tissues. α-L-iduronidase RNA expression levels were elevated in tumor samples

compared to normal adjacent tissues (Figure 1 D), indicating elevated enzyme expression in diseased tissue compared to normal tissue. This implies the possibility that an iduronidase-cleavable linker could be used for the release of an efficacious dose of the cytotoxic drug in the tumor to kill the tumor cells while limiting the general toxicity to normal, healthy tissues.

Overall, α-L-iduronidase substrate cleavage and reduced normal tissue α-L-iduronidase RNA expression suggest potential of iduronide-based ADC linkers to reduce side effects in healthy tissues.

Example 3

Synthesis of linker-payload compounds for ADC generation

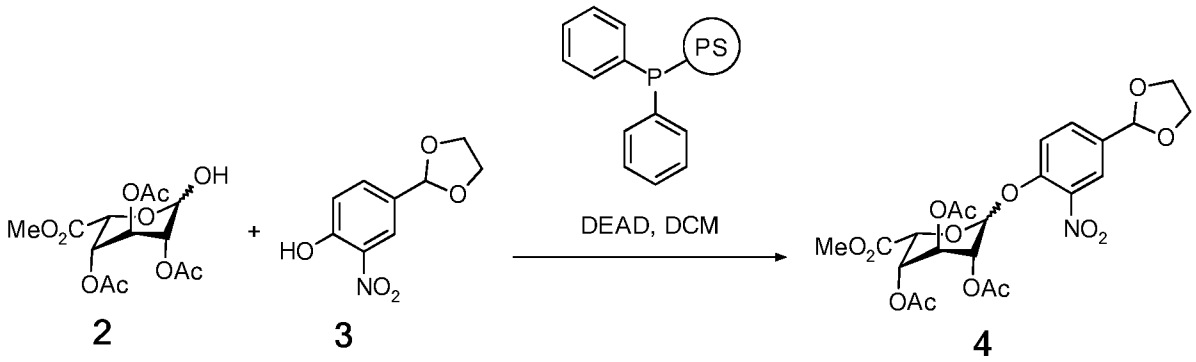
Linker-payload compounds with different warhead moieties (exatecan, duocarmycin) were synthesized (Fig. 3). Besides iduronide-based linker-payloads (compounds 12 and 15), also compounds based on the cathepsin B-cleavable peptide-linkers VC, AAN were prepared as controls (compounds 13 and 14).

The synthesis of the linker-payload MC-iduronide-exatecan (**12**) followed the scheme shown in Fig. 2.

The synthesis of the protected iduronic acid precursor (**2**) has been previously described (Lee et al., 2004; Lu et al., 2013). 4-(1,3-dioxolan-2-yl)-2-nitrophenol (**3**) was glycosylated with the protected iduronic acid precursor *via* a Mitsunobu reaction. The acetal protecting group was removed under acidic conditions and the resultant aldehyde was reduced to the primary alcohol (**5**) using sodium borohydride. In the next step, the nitro group was catalytically hydrated followed by coupling of the resultant amine to Fmoc protected glycine. To attach exatecan (**8**) to the linker portion *via* a carbamate functionality, the primary alcohol (**6**) was activated and reacted with the secondary amine of exatecan (**8**). Subsequently, acetyl, methoxy and Fmoc protecting groups were removed under basic conditions and in the last step the maleimide handle was attached to yield MC-iduronide-exatecan (**12**).

Details of the different steps of the synthesis are described in detail below.

Methyl-1-O-(4-[1,3-dioxolan-2-yl]-2-nitrophenyl)-2,3,4-tri-O-acetyl-α-L-idopyranuronate (4)

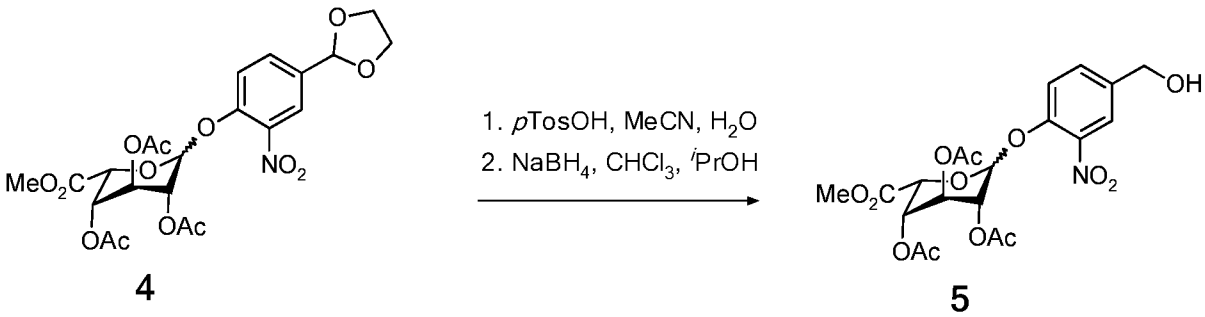


200 mg (0.598 mmol, 1 eq) hemiacetal (**2**), 190 mg (0.897 mmol, 1.5 eq) 4-(1,3-dioxolan-2-yl)-2-nitrophenol (**3**), and 299 mg polymer-bound triphenylphosphine (0. 897 mmol, 1.5 eq) were dissolved in 3 mL absolute dichloromethane under argon atmosphere and 141 µL (0.897 mmol, 1.5 eq) DEAD were added at 0 °C. After stirring for 17 h at room temperature (DC: cyclohexane/ethyl acetate 1:1), the suspension was filtered over Celite and the solvent was removed *in vacuo*. The crude substance was purified by flash chromatography (cyclohexane/ethyl acetate 15→30 %, 25 min, 25 mL/min).

yield: 201 mg (0.379 mmol, 63.4%) (**4**)

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.94 (d, ^{3}J = 2.0 Hz, 1H, CH_{Ar}), 7.64 (dd, ^{2}J = 8.7 Hz, ^{3}J = 2.0 Hz, 1H, CH_{Ar}), 7.40 (d, ^{2}J = 8.7 Hz, 1H, CH_{Ar}), 5.87 (s, 1H, H-1α), 5.80 (s, 1H, CH), 5.22-5.19 (m, 1H, H-4), 5.16-5.13 (m, 1H, H-3), 5.00-4.97 (m, 2H, H-5, H-2), 4.13-4.02 (m, 4H, CH2), 3.76 (s, 3H, OMe), 2.23 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.11 (s, 3H, OAc),
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 169.6, 169.5, 167.7 (C=O Ac), 149.1 (C_q), 133.3 (C_q), 132.4 (CH_{Ar}), 123.8 (CH_{Ar}), 116.4 (CH_{Ar}), 102.1 (CH), 95.9 (C-1α, $J_{C\text{-}1,H\text{-}1}$ = 180.1 Hz), 67.3 (C-2), 66.7 (C-4), 65.9 (C-3), 65.8 (C-5), 65.6 (CH2), 52.9 (OMe), 21.0 (Ac), 20.9 (Ac), 20.8 (Ac).

Methyl-1-O-(4-hydroxymethyl-2-nitrophenyl)-2,3,4-tri-O-acetyl-α-L-idopyranuronate (5)



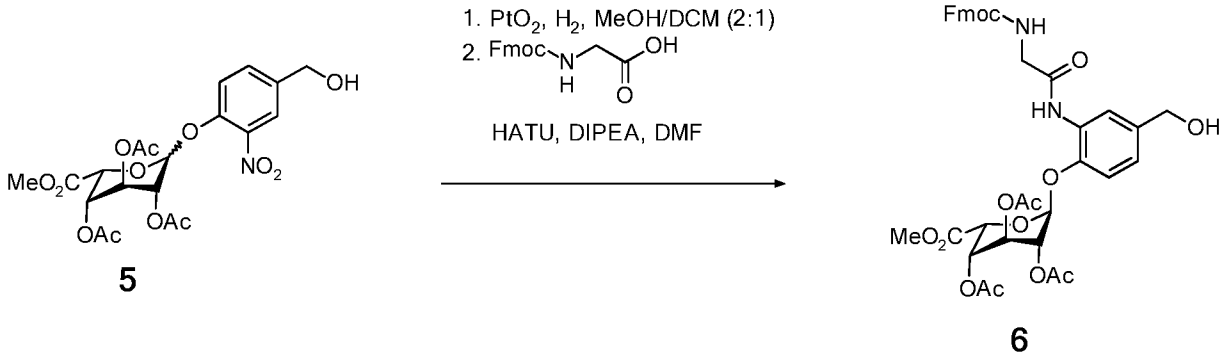
400 mg (0.759 mmol, 1 eq) iduronide (**4**) were dissolved in 80 mL dry acetonitrile. 1.15 g (6.070 mmol, 8 eq) *p*-toluenesulfonic acid monohydrate were added and the reaction solution was stirred for 30 min at room temperature. After complete conversion, the reaction was stopped by adding 489 µL (6.070 mmol, 8 eq) pyridine and the solvent was removed *in vacuo*. The residue was taken up in 80 mL of dichloromethane and extracted with 80 mL of H_2O. The organic phase was dried over magnesium sulfate and the solvent was removed *in vacuo*.

The residue was dissolved in 12.5 mL chloroform and 2.5 mL isopropanol. 37.3 mg (0.986 mmol, 1.3 eq) of sodium borohydride were added at 0 °C. After stirring for 2 h at room temperature (DC: cyclohexane/acetone 1:1), the reaction was stopped by adding 5 g of ice and the solvent was removed *in vacuo*. The residue was dissolved in 50 mL dichloromethane and extracted with 20 mL of H_2O. The organic phase was dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude substance was purified by flash chromatography (cyclohexane/ethyl acetate 40→70 %, 30 min, 25 mL/min).

yield: 245 mg (0.505 mmol, 66.5%) (**5**)

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.83 (d, ^3J = 2.0 Hz, 1H, CH_{Ar}), 7.53 (dd, ^2J = 8.7 Hz, ^3J = 2.0 Hz, 1H, CH_{Ar}), 7.37 (d, ^2J = 8.7 Hz, 1H, CH_{Ar}), 5.86 (s, 1H, H-1α), 5.22-5.20 (m, 1H, H-4), 5.16-5.13 (m, 1H, H-3), 5.01-4.98 (m, 2H, H-5, H-2), 3.78 (s, 3H, OMe), 2.24 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.11 (s, 3H, OAc),
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 169.6, 169.5, 167.8 (C=O Ac), 148.0 (C_q), 135.9 (C_q), 132.5 (CH_{Ar}), 123.8 (CH_{Ar}), 116.7 (CH_{Ar}), 96.0 (C-1α, $J_{C\text{-}1,H\text{-}1}$ = 175.5 Hz), 67.3 (C-2), 66.7 (C-4), 66.0 (C-3), 65.8 (C-5), 52.9 (OMe), 21.0 (Ac), 20.9 (Ac), 20.8 (Ac).

Methyl-1-O-(4-hydroxymethyl-2-N-[9-fluorenylmethoxycarbamate-glycin]-amino-phenyl)-2,3,4-tri-O-acetyl-α-L-idopyranuronate (6)



126 mg (0.555 mmol, 1.1 eq) PtO_2 were suspended in 2 mL absolute methanol and 1 mL absolute dichloromethane and hydrogen was added with stirring. 205 mg (0.505 mmol, 1 eq) iduronide (**5**) were dissolved in 14.4 mL absolute methanol and 7.2 mL absolute dichloromethane. The solution was added to the preactivated catalyst and stirred vigorously for 30 min under hydrogen atmosphere. After complete conversion (DC: cyclohexane/acetone 1:1), the reaction mixture was filtered over Celite and the solvent was removed *in vacuo*. The resulting amine was used for the next reaction without further purification.

150 mg (0.505 mmol, 1 eq) Fmoc-glycine and 230 mg (0.606 mmol, 1.2 eq) HATU were dissolved in 16.4 mL DMF and 171 µL (1.01 mmol, 2 eq) DIPEA were added. The solution was incubated for 10 min at room temperature and added to the amine. The reaction mixture was stirred for 18 h at room temperature. After complete conversion (DC: cyclohexane/acetone 1:1), the solvent was removed *in vacuo*. The crude substance was purified by flash chromatography (cyclohexane/acetone 25→50%, 30 min, 30 mL/min).

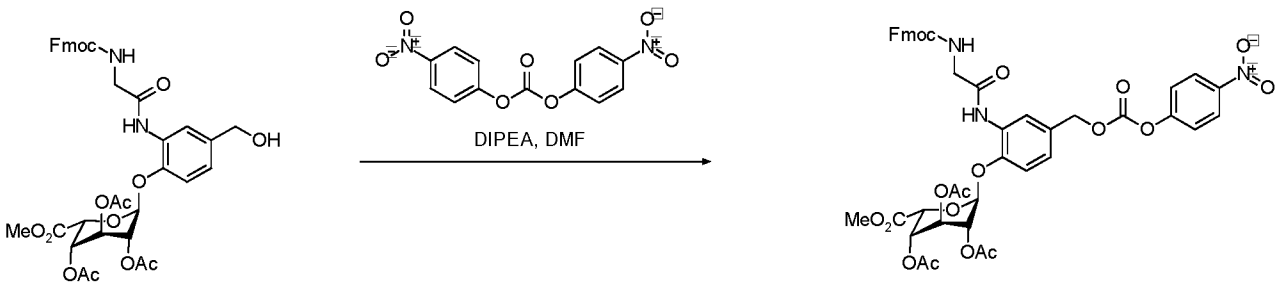
yield: 255 mg (0.347 mmol, 68.8 %) (**6**)

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.35-8.29 (m, 2H, CH_{Ar}), 7.76 (d, J = 7.7 Hz, 2H, CH_{Ar}), 7.63 (d, J = 7.7 Hz, 2H, CH_{Ar}), 7.42-7.36 (m, 1H, CH_{Ar}), 7.34-7.21 (m, 2H, CH_{Ar}), 5.80-5.73 (m, 1H, H-1α), 5.42-5.36 (m, 1H, H-4), 5.30-5.20 (m, 2H, H-3, H-2), 5.00 (d, $J^{4,5}$ = 4.7 Hz, 1H, H-5), 4.65 (s, 2H, CH_2), 4.48-4.36 (m, 2H, CH_2), 4.25 (t, J = 6.8 Hz, 1H, CH), 3.81 (s, 3H, OMe), 2.17 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc),

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 169.6, 169.5, 167.8 (C=O Ac), 148.0 (C_q), 135.9 (C_q), 127.9, 127.1, 125.3, 123.3, 120.1, 119.5, 115.1 (CH_{Ar}), 97.7 (C-1α, $J_{C\text{-}1,H\text{-}1}$ = 175.5 Hz), 69.7 (C-5),

69.7 (C-3), 68.4 (C-2), 67.9 (C-4), 67.3 (CH_2), 64.9 (CH_2), 52.8 (OMe), 47.2 (CH), 21.0 (Ac), 20.8 (Ac), 20.6 (Ac).

Methyl (3S,4R,6S)-3,4,5-tris(acetyloxy)-6-{2-[2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)acetamido]-4-(hydroxymethyl)phenoxy}oxane-2-carboxylate (7)

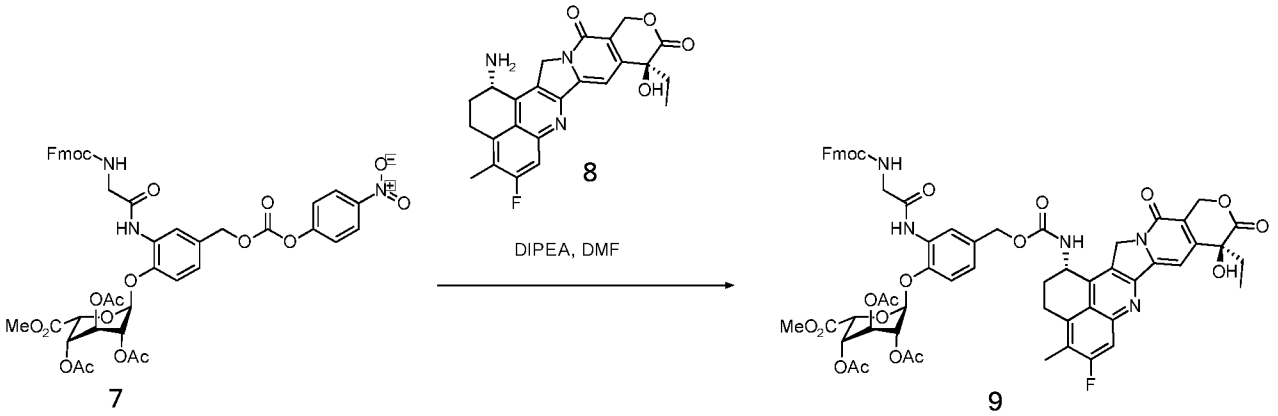


6 7

150 mg (0.20 mmol, 1 eq) iduronide (**6**) were dissolved in DMF (3.0 mL). To this solution, 122.4 mg (0.40 mmol, 2 eq) bis(4-nitrophenyl) carbonate and 102.6 µL DIPEA (0.60 mmol, 3 eq) were added. The reaction mixture was stirred at ambient temperature for 3.5 h and purified by flash chromatography.

yield: 116 mg (0.13 mmol, 64.0%) (**7**)

(3S,4R,6S)-6-{4-[({[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1(24),2(14),6(11),12,15,17,19-heptaen-23-yl]carbamoyl}oxy)methyl]-2-[2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)acetamido]phenoxy}-3,4,5-trihydroxyoxane-2-carboxylic acid (9)



7 8 9

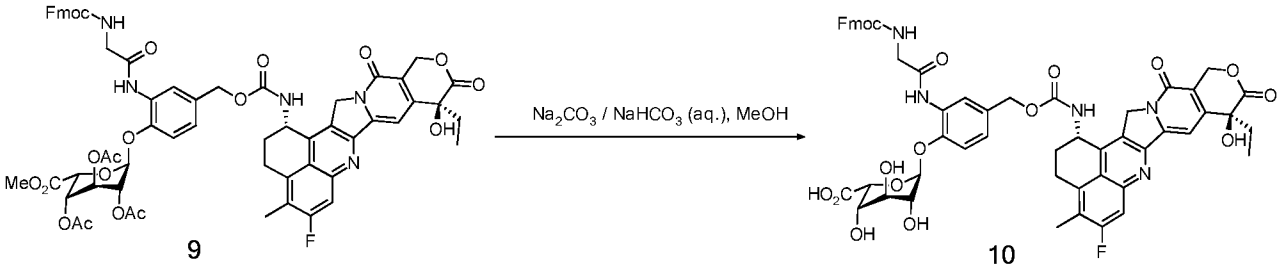
116 mg (0.13 mmol, 1 eq) iduronide (**7**) were dissolved in 3 mL DMF. 68.4 mg (0.13 mmol, 1 eq) exatecan mesylate (**8**) were added. To this suspension, 43.7 µL (0.26 mmol, 2 eq) DIPEA

were added, and the resulting solution was stirred at room temperature for 16 h. The solvent was removed *in vacuo* and the crude substance was purified by flash chromatography.

yield: 120 mg (0.10 mmol, 75.2%) (**9**)

MS (positive mode): 1195.37 (**9**; exact mass), 1195.4 (observed)

(3S,4R,6S)-6-{4-[({[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1(24),2(14),6(11),12,15,17,19-heptaen-23-yl]carbamoyl}oxy)methyl]-2-[2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)acetamido]phenoxy}-3,4,5-trihydroxyoxane-2-carboxylic acid (10)

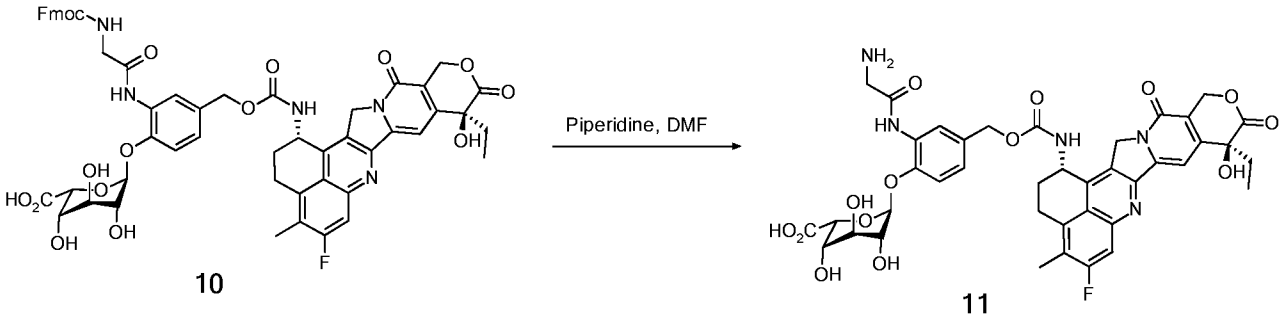


120 mg (0.1 mmol, 1 eq) iduronide (**9**) were suspended in 5.0 mL methanol and a saturated Na_2CO_3/ $NaHCO_3$ solution (9:1, pH 10.8, 2.5 mL) was added dropwise within 30 min. At this step it was important that the pH did not exceed pH 10 as this would lead to decarboxylation at the iduronide. After the addition of base was finished, the yellow suspension was stirred for 2.5 h at ambient temperature to complete deprotection. The reaction was stopped by the addition of citric acid (pH 6.0) and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography yielding the desired compound (**10**) and the Fmoc deprotected compound (**11**).

yield: 46 mg (0.04 mmol, 44.2%) (**10**)

MS (positive mode): 1055.32 (**10**; exact mass), 1056.5 (observed)

(3S,4R,6S)-6-[2-(2-aminoacetamido)-4-[({[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamoyl}oxy)methyl]phenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid (11)

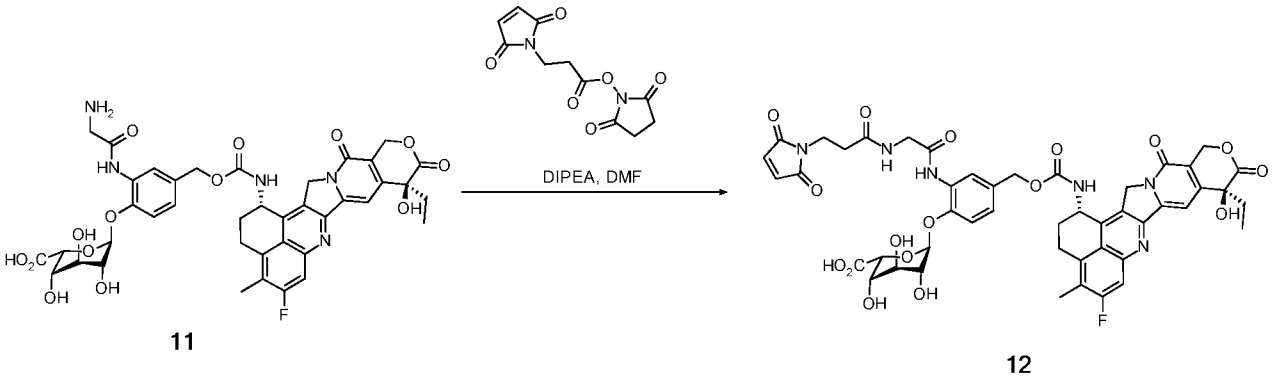


46 mg (0.04 mmol, 1 eq) iduronide (**10**) was dissolved in 3.0 mL DMF. To this solution, 20 µL (0.20 mmol, 4.6 eq) piperidine were added and the mixture was stirred at ambient temperature for 30 min. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography.

yield: 33.5 mg (0.04 mmol, 91.6%) (**11**)

MS (positive mode): 833.26 (**11**; exact mass), 833.7 (observed)

(3S,4R,6S)-6-(2-{2-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido]acetamido}-4-[({[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamoyl}oxy)methyl]phenoxy)-3,4,5-trihydroxyoxane-2-carboxylic acid (12)



33.5 mg (0.04 mmol, 1 eq) iduronide (**11**) were dissolved in 2.0 mL DMF. To this solution, 13.57 µL (0.08 mmol, 2 eq) DIPEA and 10.6 mg (0.04 mmol, 1eq) 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate were added. The yellow solution was stirred

at ambient temperature for 1 h before the solvent was removed *in vacuo* and the crude substance was purified by flash chromatography.

yield: 32.5 mg (0.03 mmol, 70.2%) (**12**)
MS (positive mode): 984.28 (**12**; exact mass), 984.5 (observed)

Compound 15 (iduronide-duocarmycin) was synthesized via a very similar route as compound 12.

Example 4

Profiling of iduronidase-mediated iduronide drug-linker cleavage in direct comparison with peptide-based Cathepsin B-mediated cleavage

The susceptibility of the iduronidase linker to enzymatic cleavage was evaluated by treatment of the cysteine adduct of compound **12** (**16**) with recombinant α-L-iduronidase (Figure 4A-C). To identify pH optima for recombinant α-L-iduronidase and recombinant cathepsin B as reference control, an HPLC assay at different pH levels was applied to monitor payload release rates from cysteine adducts of the iduronidase linker (**16**) or peptide linker (**cys-13**), respectively (Figure 4C). Correlation of applied pH with the cleavage rates (K_{enz} [1/h]) indicated pH 4.5 and 5.0 as pH optima for α-L-iduronidase and recombinant cathepsin B, respectively. Next, an HPLC assay was applied to monitor the loss of the iduronide moiety (**17, "intermediate"**) and release of the free warhead (**18, exatecan**) (Figure 4A) at 37 °C and pH 4.5 (Figure 4C). Similarly, the cysteine adduct of a peptide linker (**derivative of 13**) was monitored during cleavage with recombinant cathepsin B at pH 5.0. The cleavage of iduronide from 16 resulted in the rapid formation of the exatecan intermediate (**17**) from which exatecan (**18**) was released in a following step by 1,6 elimination (**18**), as confirmed by LC-MS. Interestingly, an exatecan intermediate (**17**) was rapidly formed upon iduronide cleavage followed by rapid 1,6 elimination of exatecan (Figure 4, B). In a similar experiment with peptide linker cysteine adduct (**cys-13**) and cathepsin B treatment, no exatecan intermediate, but only the direct formation of free exatecan could be detected by LC-MS (**18**) with similar release kinetics when compared to the iduronide linker. Overall, it was demonstrated that the iduronide linker is a substrate for the recombinant α-L-iduronidase suggesting that the linker is likely also a substrate for the human lysosomal α-L-iduronidase. Moreover, it was shown that the payload was rapidly liberated upon iduronidase-mediated hydrolysis.

Example 5

Profiling of iduronidase-mediated drug-linker cleavage in direct comparison with cathepsin B-mediated cleavage

Uptake of intact ADCs into normal cells was described as main driver of ADC toxicity. ADCs enter lysosomal trafficking followed by the release of payloads from the ADC inside of non-targeted healthy cells, potentially leading to unwanted toxicities (Nguyen et al., 2023). Moreover, it was described that the linker component can contribute to ADC-induced toxicities as the lysosomal payload release rate impacts the concentration of free payload (Colombo and Rich, 2022).

To explore whether iduronidase-mediated payload release could be reduced compared to legumain- (another peptidase which can be leveraged for prodrug cleavage) and/or cathepsin B-mediated release, payload liberation profiles were monitored in cell lysates or lysosomal extracts from different human normal cell types that served as model systems for tissues or cells often affected by ADC toxicity including hematopoietic cells (e.g. cells of myeloid lineage) or hepatocytes (Masters et al., 2018) (Figure 5 A and B). Cysteine adducts of iduronidase- (**16**) or peptide-linkers (**cys-13 or cys-14**) were applied in cell lysates from mononuclear cells (MNC), peripheral blood mononuclear cells (PBMC), normal human dendritic cells (NHDC), normal human epidermal keratinocytes (NHEK), human umbilical vein (HUVEC) cell or liver lysosomes. Payload (**18, exatecan**) liberation profiles were obtained for up to 48 h from quantification by LC-MS/MS and calculated as %total exatecan (5 µM = 100%).

Interestingly, enhanced exatecan release from MC-K(Ac)VC-exatecan or MC-AAN-exatecan (cleavable by the enzyme legumain) compared to MC-iduronide-exatecan was observed which reflects reduced iduronidase concentration in cell lysates and lysosomal extracts (Figure 5 A and B). Moreover, these findings indicate that iduronidase-mediated payload release could be reduced in associated cell types and hence ADCs equipped with iduronidase-cleavable linkers may have the potential to reduce on-target toxicity effects.

Example 6

Generation and stability assessment of peptide- and iduronid-based conjugates, exemplarily described for anti-CEACAM5(aCEA5)-based ADCs

The ADC preparation with **12** (Figure 6A), **13**, **14** was conducted as previously described (Jaeger et al. 2021; Kaempffe et al., 2021). Conjugation of MC-iduronide-duocarmycin (**15**) was conducted as previously described in Dickgiesser et al., 2020. Conjugation reactions resulted in homogenous ADCs of high purity (SEC (size exclusion chromatography) purities ranging between 93.6%-99.8% (Figure 6B)) with DARs ranging from 7.7 to 8.0 for conjugation of the interchain cysteines or a DAR of 2 for the MTG-catalyzed conjugations (Figure 6 B).

For the produced ADCs, the apparent hydrophobicity was further analyzed using HIC (Figure 6 B). Interestingly, all ADC variants exhibited similar relative retention times (RRTs) by hydrophobic interaction chromatography (HIC) in the range of 1.05−1.38 when compared to ADCs equipped with Iduronid-based linkers (1.05 and 1.09), whereas ADCs equipped with peptide-based linkers showed higher HIC RRTs (1.22 and 1.38).

Several studies have shown that conjugate pharmacokinetics and efficacy can strongly be influenced by serum stability (Dorywalska et al., 2015; Shen et al., 2012). Therefore, ADC stability was assessed in mouse, human and cynomolgus serum, followed by free payload determination using LC-MS/MS. Only minor amounts of payload (below 1% of total calculated conjugated payload) were released within 96 h of incubation indicating high *in vitro* serum stability for tested iduronide-ADC (Figure 6 C).

Example 7

Iduronide-based and petide-based aCEA5-exatecan conjugates can specifically kill cancer cells in vitro with high potency

Human cancer cell lines were used to assess the potential to kill cancer cells of aCEA5-iduronide-exatecan and aCEA5-K(Ac)VC-exatecan along with free exatecan as control. Both aCEA5-iduronide-exatecan and aCEA5-K(Ac)VC-exatecan ADCs showed sub-nanomolar *in vitro* potency against different CEACAM5-positive (SK-CO-1, MKN-45, LS174T) and minor effect on CEACAM5-negative cell lines (MDA-MB-231) (Figure 7B). As shown in the

respective dose-response curves (Figure 7 A), aCEA5-iduronide-exatecan and aCEA5-K(Ac)VC-exatecan were very potent against CEACAM5-positive cell lines SK-CO-1, MKN-45 and LS174T. In contrast, effects of aCEA5-iduronid-exatecan and aCEA5-K(Ac)VC-exatecan on antigen-negative MDA-MB-231 were limited to the highest concentrations tested (Figure 7 A).

In conclusion, aCEA5-iduronide-exatecan specifically kills CEACAM5 expressing human cancer cell lines *in vitro* with high potency.

Example 8

In vitro serum stability and in vitro killing of cancer cells by iduronide-based aEGFR-exatecan conjugates

Characterization of iduronide-based conjugates included also the assessment of the stability in mouse serum followed by free payload determination using LC-MS/MS. Only minor amounts of payload (below 1% of total calculated conjugated payload) were released within 96 h of incubation indicating high *in vitro* serum stability for tested iduronide-ADC (Figure 8 A).

Human cancer cell lines were used to assess the potential of aEGFR(anti-EGFR)-iduronide-exatecan and aEGFR-K(Ac)VC-exatecan to kill cancer cells, along with free exatecan as control. Both, the aEGFR-iduronide-exatecan and aEGFR-K(Ac)VC-exatecan ADCs showed sub-nanomolar *in vitro* potency against EGFR-positive MDA-MB-468 and minor effects on the EGFR-negative cell line A673 (Figure 8 B). As shown in dose-response curves (Figure 8 B), aEGFR-iduronide-exatecan and aEGFR-K(Ac)VC-exatecan were potent against the EGFR-positive cell line MDA-MB-468. In contrast, effects of aEGFR-iduronide-exatecan and aEGFR-K(Ac)VC-exatecan on antigen-negative MDA-MB-231 were limited to the highest concentrations tested (Figure 8). In conclusion, aEGFR-iduronide-exatecan specifically kills EGFR expressing human cancer cell lines *in vitro* with high potency, indicating broad applicability of the iduronide-based conjugates.

Example 9

In vitro exatecan release in cell lysates.

In most patients which undergo anti-EGFR therapy, dermatological on-target toxicity side effects occur, which takes the form of skin rashes and can impact the patient's adherence to the therapy regime (Fakih et al., 2010). Side effects arise from effects on EGFR expressing keratinocytes in the basal layer in the epidermis, ultimately leading to various morphological effects like papulopustular eruption or xerosis, telangiectasias and pruritus.

To evaluate the ADC payload linker cleavage in normal epidermal cells, cell lysates from primary normal human epidermal keratinocytes (NHEK) were chosen as model system (Figure 9). Payload liberation profiles for unconjugated iduronide-based structures compared to a peptide-based exatecan linker were obtained by calculating free exatecan (initial conc. ~10 µM exatecan) from normalized data. Interestingly, pronounced payload release was observed for peptide-based exatecan linker mediated payload liberation (~100% of initial total payload linker), while almost no payload release was observed for the iduronide-based structure (<1% of initial total payload linker) (Figure 9).

In conclusion, greatly reduced payload liberation from iduronide-based structures in normal epidermal cells indicate a potential to reduce on-target toxicity effects for EGFR-directed ADCs.

Example 10

An iduronide-based ADC conjugate can effectively kill cancer cells in vitro with high potency

Iduronide-based aCEA5 duocarmycin conjugates were synthesize and successfully characterized *in vitro*, demonstrating the broad applicability of iduronide-based linkers. The CEA5-positive human cancer cell line MKN45 and the corresponding CEA5-negative MKN45-CEA5 knock-out cell lines were used to assess the potential of aCEA5-iduronide-duocarmycin along with free duocarmycin as control to kill cancer cells. The aCEA5-iduronide-duocarmycin ADC showed nanomolar *in vitro* potency against CEACAM5-positive MKN-45 cells and minor effect on CEACAM5-negative knock-out cells (MKN-45 CEA knock-out) (Figure 10). As shown in the respective dose-response curves (Figure 10), aCEA5-iduronide-duocarmycin

ADC was very potent against the CEACAM5-positive cell line MKN-45. In contrast, effects of aCEA5-iduronide-duocarmycin ADC on antigen-negative MKN-45 CEA knock-out cells were limited to the highest concentrations tested (Figure 10).

In conclusion, the resulting aCEA5-iduronide-duocarmycin ADC specifically killed CEACAM5 expressing human cancer cell lines *in vitro* with high potency, indicating potential broad applicability of the iduronide-based conjugates.

Example 11

Cell killing effects of aCEA5-iduronide-exatecan in mono- and co-cultures

To explore if reduced iduronidase mRNA expression (Figure 1) and reduced payload release profiles (Figure 5) may translate into beneficial reduction of cell killing effects, antigen-negative primary hepatocytes and liver sinusoidal endothelial cells (LSECs) were used along with the aCEA5-iduronide-exatecan ADC in cell viability assays.

The potential of the aCEA5-iduronide-exatecan construct to mediate reduced cell killing effects was compared to peptide-linker-based exatecan ADCs including aCEA5-K(Ac)VC-exatecan and aCEA5-AAN-exatecan, along with free exatecan as a control (Figure 11, A and B).

The aCEA5-iduronide-exatecan ADC showed reduced cell killing effects on mono- and co-cultured LSECs/hepatocytes and HUVESs, compared to the peptide-linker-based exatecan ADCs. These findings demonstrate that iduronide-based linkers can reduce toxicity effects on antigen-negative normal cells.

Example 12

Efficacy of an iduronide-based ADC in a SNU-16 gastric cancer (GC) xenograft (CDX) mouse model

Anti-tumor efficacy *in vivo* has been evaluated in the human gastric carcinoma xenograft model SNU-16. Six to eight week old immunodeficient female mice (C;129P2-H2d-TgH(Il2rg)tm1Bm-TgH(Rag2)tm1AltN4) were injected subcutaneously in the right flank with 5 million SNU-16 cells. When tumors reached a mean volume of 139 mm^3 (in the range of 105-

201 mm³, n=10 per treatment arm) mice were treated once intravenously with vehicle (saline solution; 5 ml/kg) or with aCEA-duocarmycin ADC (5 mg/kg; 5 ml/kg). Tumor length (L) and width (W) were measured with calipers and tumor volumes were calculated using L×W^2/2. Tumor size and body weight have been investigated twice per week. The single treatment with aCEA-duocarmycin ADC at a dose of 5 mg/kg led to a significant anti-tumor effect (Figure 12). Treatment with aCEA-duocarmycin ADC had no significant impact on body weight (data not shown).

Example 13

Evaluation of the effect of an iduronidase-cleavable ADC on human neutrophil differentiation

The neutrophil differentiation assay is performed to verify that an iduronidase-cleavable aCEA-duocarmycin ADC results in reduced toxicity effects on neutrophil cells, compared to an aCEA5-VC-duocarmycin ADC. The assay is performed according to Zhao et al., 2017 including the minor variations that X-Vivo serum free medium (ReachBio) and mobilized peripheral blood CD34+ cells are used. In that manner, CD34+ cells (10,000 per well of a round bottom 96-well plate) are set up in a media formulation containing X-Vivo medium with rhFLT-3 ligand, rhSCF, rhIL-3 and rhIL-6 for 3 days. The plates are then washed and new X-Vivo medium containing rhFLT-3 ligand, rhSCF, rhIL-3, rhGM-CSF and rhG-CSF is applied for 4 days. The plates are then washed again and the cells are resuspended in X-vivo containing rhIL-3 and rhG-SCF for an additional 4 days. The plates are then washed one last time and the cells are resuspended in X-Vivo medium containing just rhG-CSF. At this time point, iduronidase-cleavable aCEA-duocarmycin ADC or aCEA5-VC-duocarmycin ADC or free duocarmycin payload as control are added for 6 days (6 concentrations in triplicate). Appropriate solvent controls are included as well. The percentage and number of CD66b+ cells are analyzed by flow cytometry.

In comparison to the aCEA5-VC-duocarmycin ADC, the iduronidase-cleavable aCEA-duocarmycin ADC shows decreased *in vitro* potency against neutrophil cells indicating the potential to reduce undesired toxicity side effects on cells of the neutrophil cell lineage.

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CLAIMS

1. A compound having a structure according to the formula

$$\text{Ligand}\text{-}(\text{-}A_a\text{-}W_w\text{-}Y_y\text{-}F_f)_p \qquad \text{(Formula 5)},$$

wherein:

Ligand is a Ligand unit;

A is a Stretcher unit;

a is 0, 1 or 2;

W is an Iduronide unit;

w is 1 or 2;

Y is a Spacer unit;

y is 0, 1 or 2;

A_a–W_w–Y_y is a Linker unit;

F is a Functional agent;

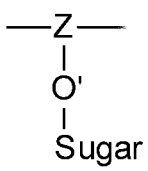
f is 1, 2, 3 or 4;

p is from 1 to 20.

2. The compound according to claim 1, wherein said Iduronide unit is a chemical group that comprises an iduronide and an iduronidase cleavage site.

3. The compound according to any one of claims 1 or 2, wherein the Iduronide unit undergoes self-immolation upon cleavage at the iduronidase cleavage site.

4. The compound according to any one of claims 1 to 3, wherein said Iduronide unit (W) comprises a sugar moiety ("Sugar") linked via a glycosidic bond (–O'–) to a self-immolative group (Z), according to the formula:



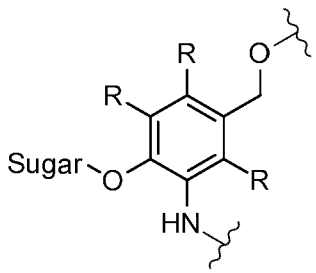
(Formula 9),

wherein said glycosidic bond (–O'–) is an iduronidase cleavage site.

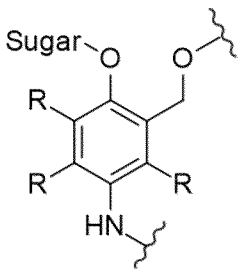
5. The compound according to claim 4, wherein the sugar moiety is an iduronide moiety.

6. The compound according to any one of claims 4 or 5, wherein, upon cleavage of the glycosidic bond that links said self-immolative group to said sugar moiety, said self-immolative group decomposes in such a way that the covalent link between said Functional agent and said Ligand unit is disrupted and the Functional agent is released.

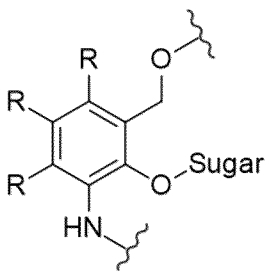
7. The compound according to any one of claims 1 to 6, wherein the Iduronide unit has a structure according to a formula selected from the group consisting of the following formulas:



(Formula 20)



(Formula 21)



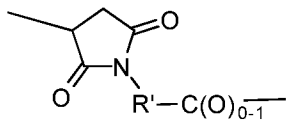
(Formula 22),

wherein

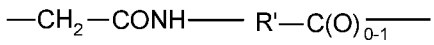
Sugar is an iduronide; and

each R is independently selected from the group consisting of hydrogen, halo, —CN, —NO_2, and another electron-withdrawing or electron-donating group, provided that the Iduronide unit undergoes self-immolation upon cleavage of the glycosidic bond.

8. The compound according to any one of claims 1 to 7, wherein the Stretcher unit has a structure according to a formula selected from the group consisting of the following formulas:



(Formula 34)



(Formula 35),

wherein R' is a direct bond or selected from the group consisting of C_1-C_{10} alkylene, C_3-C_8 carbocyclo, –O–(C_1-C_8 alkyl), arylene, C_1-C_{10} alkylene-arylene-, arylene-C_1-C_{10} alkylene, C_1-C_{10} alkylene (C_3-C_8 carbocyclo), (C_3-C_8 carbocyclo) –C_1-C_{10} alkylene-, C_3-C_8 heterocyclo-, –C_1-C_{10} alkylene-(C_3-C_8 heterocyclo)-, (C_3-C_8 heterocyclo) –C_1-C_{10} alkylene, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, and –$(CH_2CH_2O)_r$–CH_2–CH_2–; and r is an integer ranging from 1-10.

9. The compound according to any one of claims 1 to 8, wherein the Spacer unit is a meta-aminobenzyl alcohol unit whose phenylene portion is substituted with Q_m, wherein Q is selected from the group consisting of –(C_1-C_8 alkyl), –O–(C_1-C_8 alkyl), –halogen, –nitro and –cyano; and *m* is 0, 1, 2, 3 or 4.

10. The compound according to any one of claims 1 to 9, wherein said Ligand unit (L) comprises an antibody.

11. The compound according to any one of claims 1 to 10, wherein said Functional agent is a therapeutic agent or a detectable label.

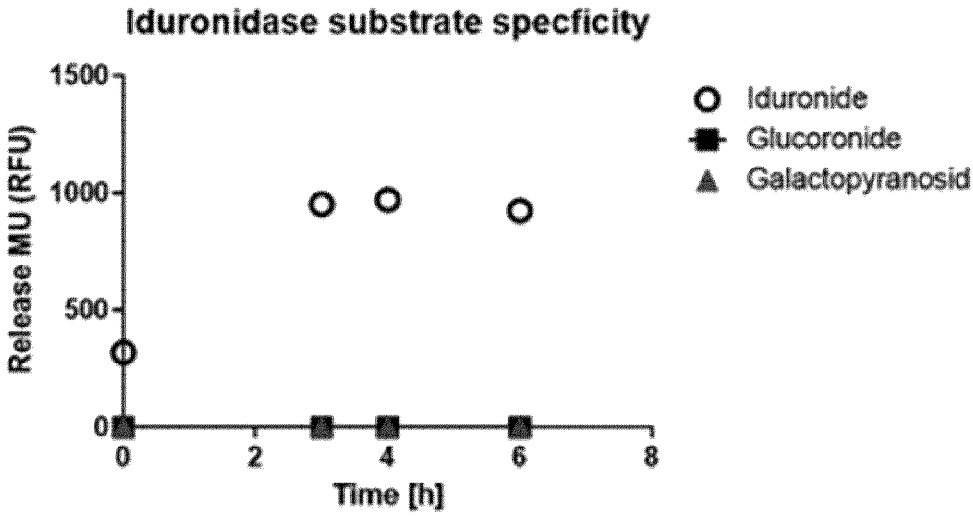
12. A method for preparing a compound according to any one of claims 1 to 11, wherein said method comprises the step of covalently linking at least one molecule comprising a Functional agent to a molecule comprising a Ligand unit.

13. Use of a molecule in the preparation of a compound according to any one of claims 1 to 11, wherein said molecule comprises an Iduronide unit covalently linked to an activator group.

14. A method for increasing the cytotoxicity of a molecule, said molecule comprising a Ligand unit, wherein said method comprises covalently linking at least one Functional agent to said molecule, thus providing a compound according to any one of claims 1 to 11, wherein said Functional agent is a cytotoxic agent.

15. The compound according to any one of claims 1 to 11 for use in the treatment of cancer, wherein said Functional agent is a cytotoxic agent.

A


Iduronidase substrate specficity
Release MU (RFU)
1500
1000
500
0
0
2
4
6
8
Time [h]
Iduronide
Glucoronide
Galactopyranosid

B

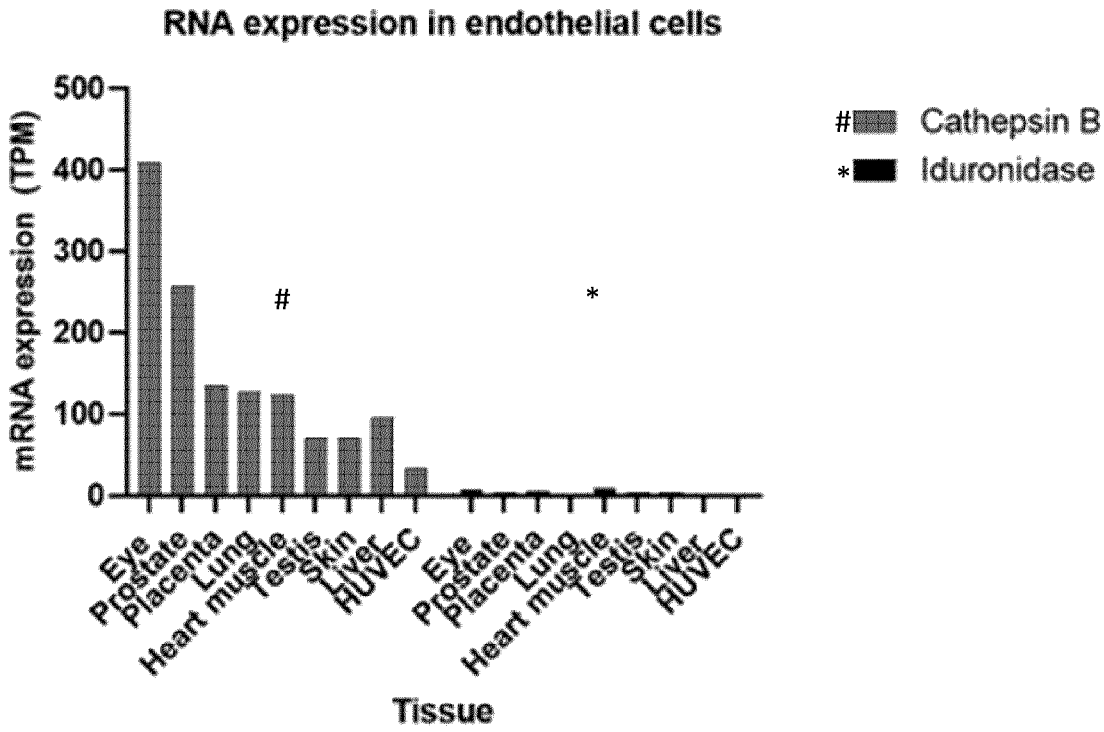
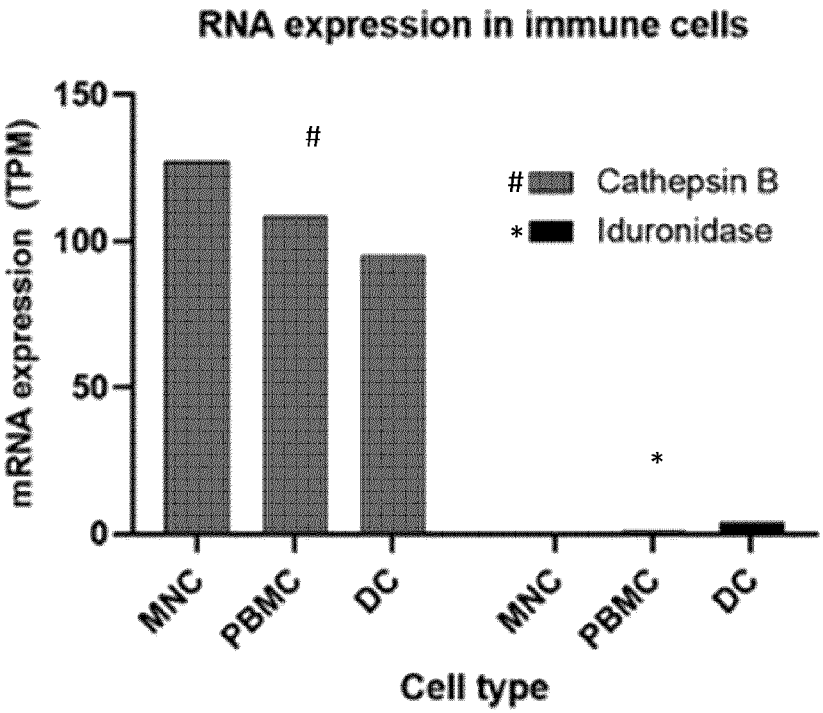

RNA expression in endothelial cells
mRNA expression (TPM)
500
400
300
200
100
0
#
*
Eye
Prostate
Placenta
Lung
Heart muscle
Testis
Skin
Liver
HUVEC
Tissue
Cathepsin B
* Iduronidase

Fig. 1 A, B

C


RNA expression in immune cells
mRNA expression (TPM)
150
100
50
0
#
*
Cathepsin B
* Iduronidase
MNC
PBMC
DC
MNC
PBMC
DC
Cell type

D

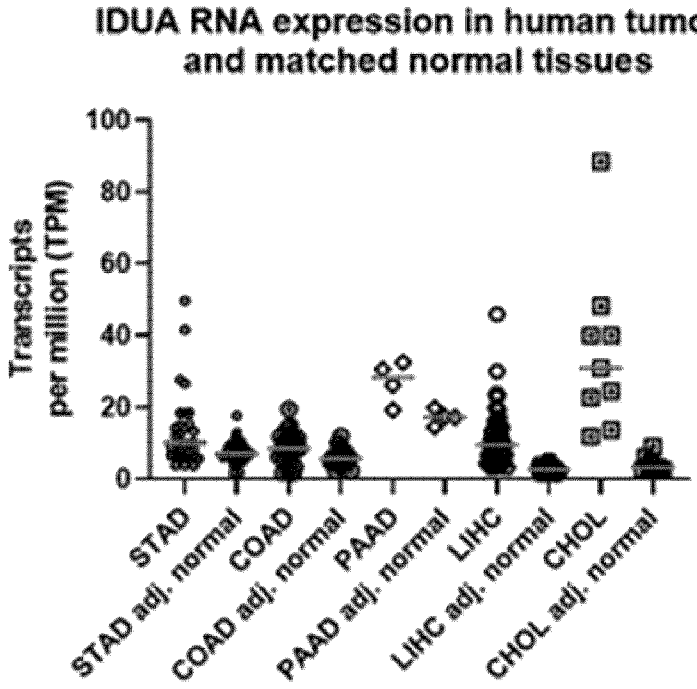

IDUA RNA expression in human tumors
and matched normal tissues
Transcripts
per million (TPM)
100
80
60
40
20
0
STAD
STAD adj. normal
COAD
COAD adj. normal
PAAD
PAAD adj. normal
LIHC
LIHC adj. normal
CHOL
CHOL adj. normal

Fig. 1 C, D

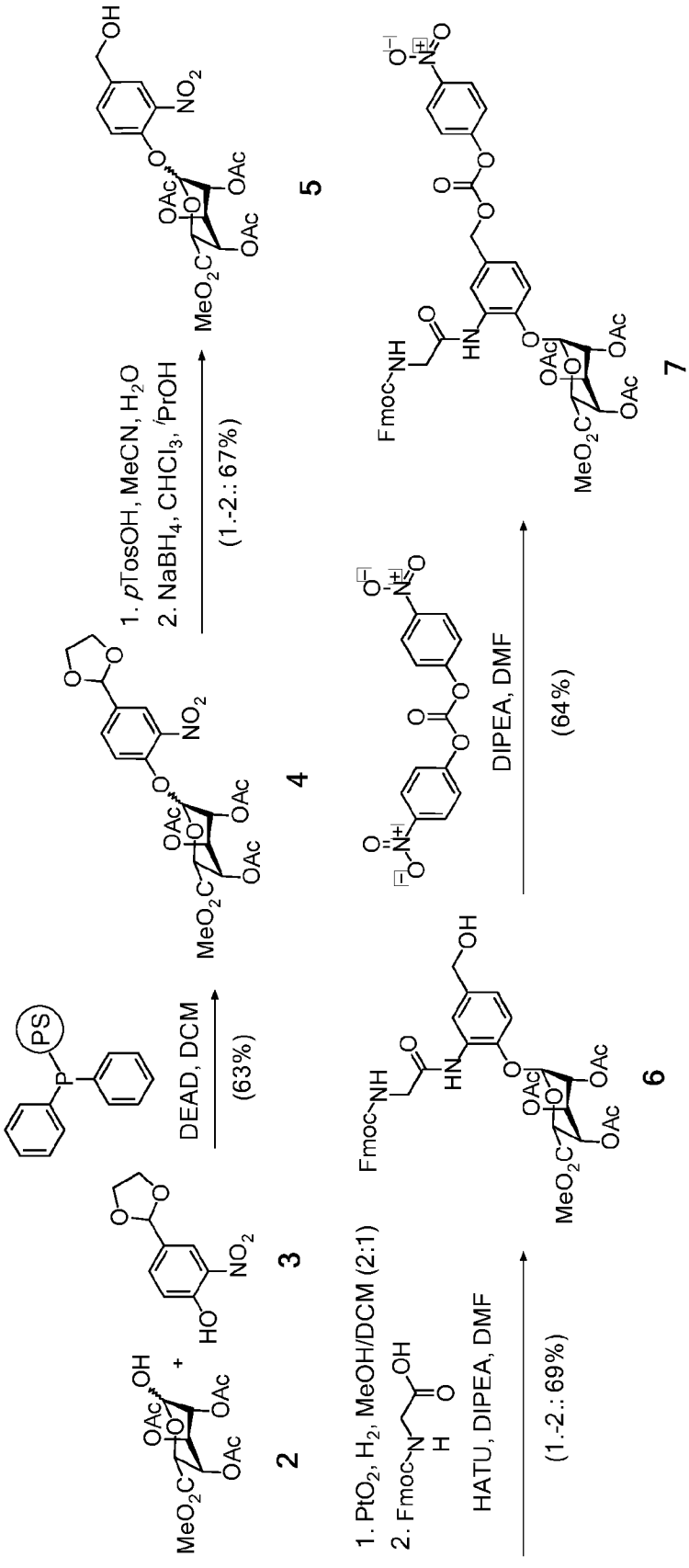

1. PtO2, H2, MeOH/DCM (2:1)
2. Fmoc-N(H)-CH2-C(=O)-OH
HATU, DIPEA, DMF
(1.-2.: 69%)
DEAD, DCM
(63%)
1. pTosOH, MeCN, H2O
2. NaBH4, CHCl3, iPrOH
(1.-2.: 67%)
DIPEA, DMF
(64%)
PS
2
3
4
5
6
7

Fig. 2 A

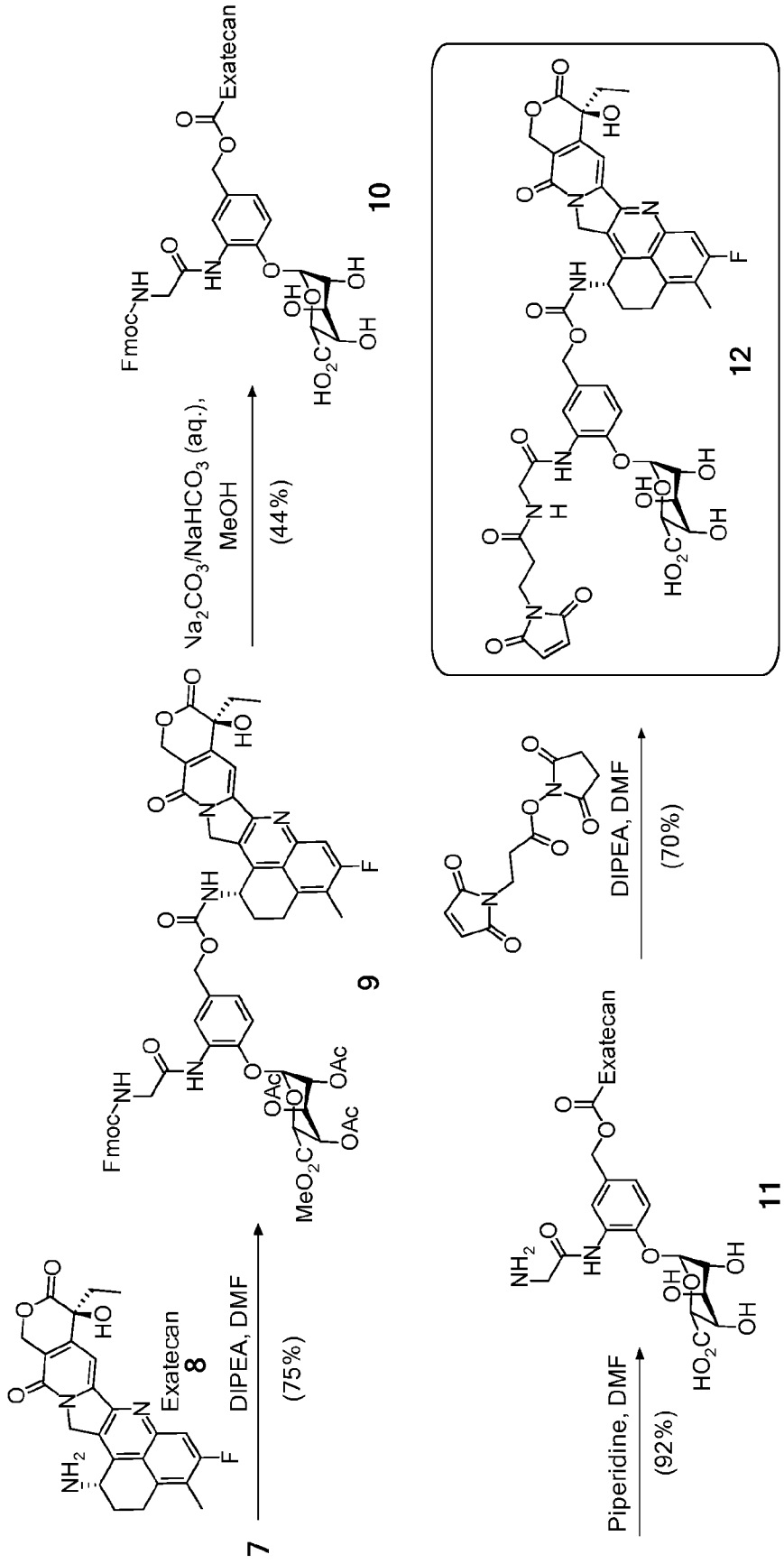

Exatecan
8
DIPEA, DMF
(75%)
7
9
Va2CO3/NaHCO3 (aq.),
MeOH
(44%)
10
DIPEA, DMF
(70%)
12
Piperidine, DMF
(92%)
11

Fig. 2 B

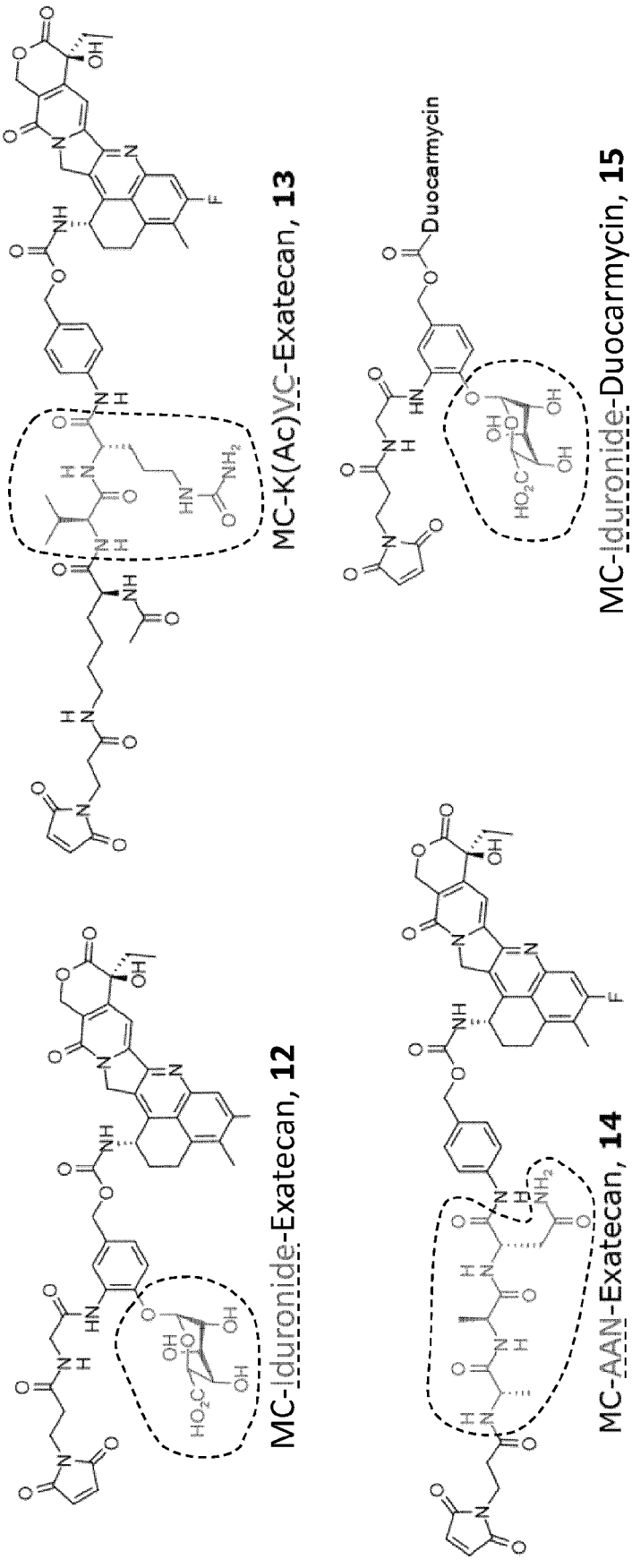

MC-K(Ac)VC-Exatecan, 13
MC-Iduronide-Duocarmycin, 15
MC-Iduronide-Exatecan, 12
MC-AAN-Exatecan, 14

Fig. 3

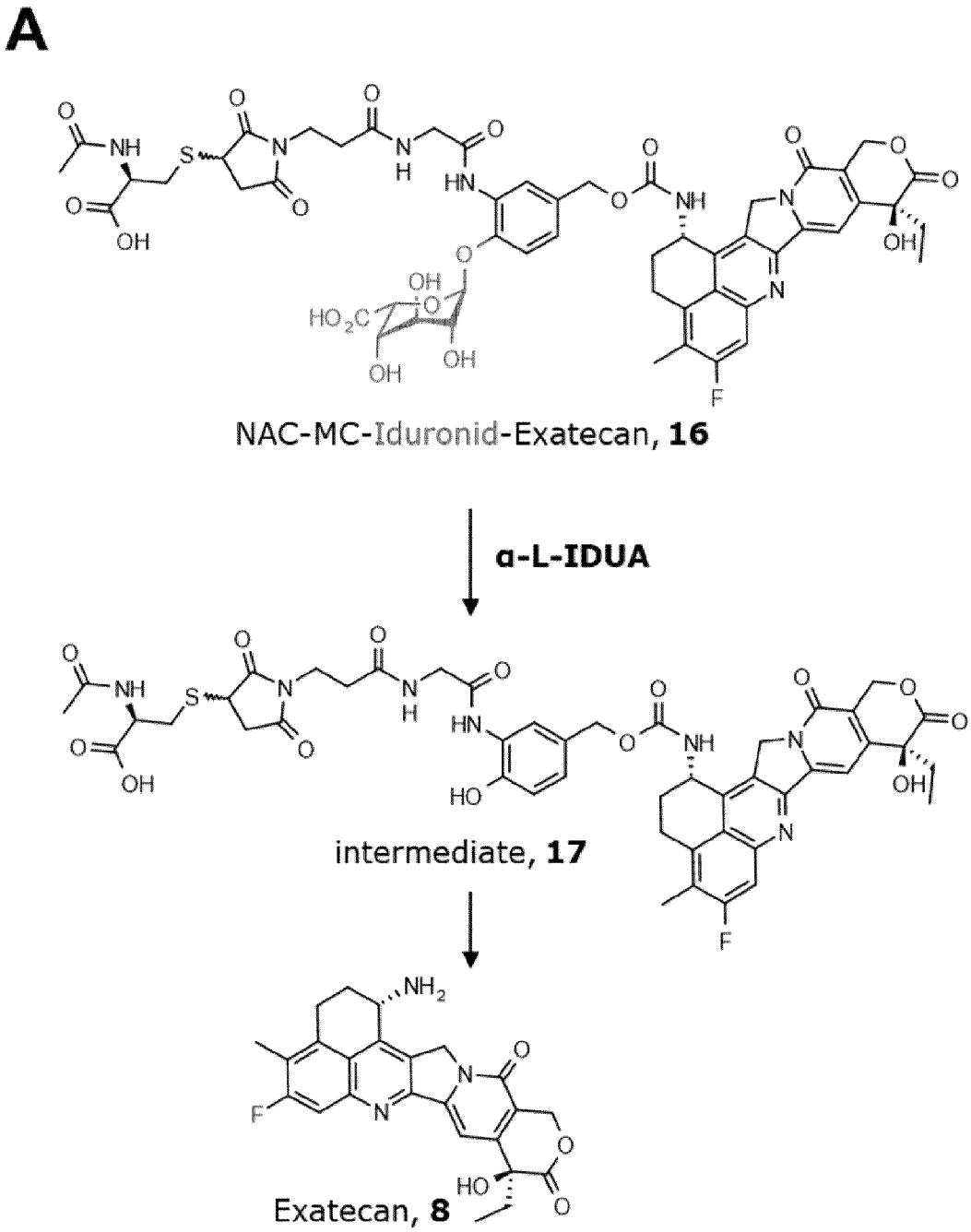

A
NAC-MC-Iduronid-Exatecan, 16
α-L-IDUA
intermediate, 17
Exatecan, 8

Fig. 4 A

B

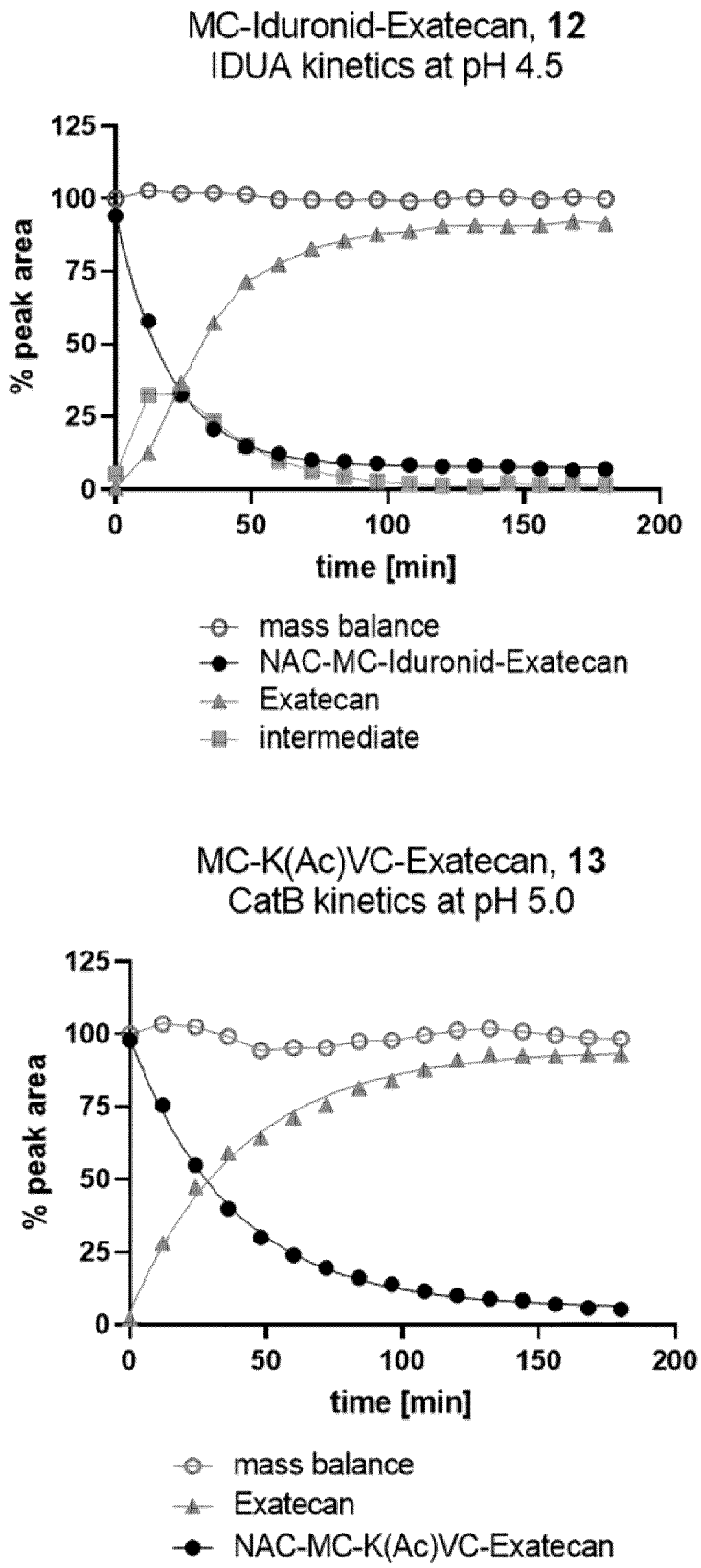

MC-Iduronid-Exatecan, 12
IDUA kinetics at pH 4.5
% peak area
time [min]
mass balance
NAC-MC-Iduronid-Exatecan
Exatecan
intermediate
MC-K(Ac)VC-Exatecan, 13
CatB kinetics at pH 5.0
% peak area
time [min]
mass balance
Exatecan
NAC-MC-K(Ac)VC-Exatecan

Fig. 4 B

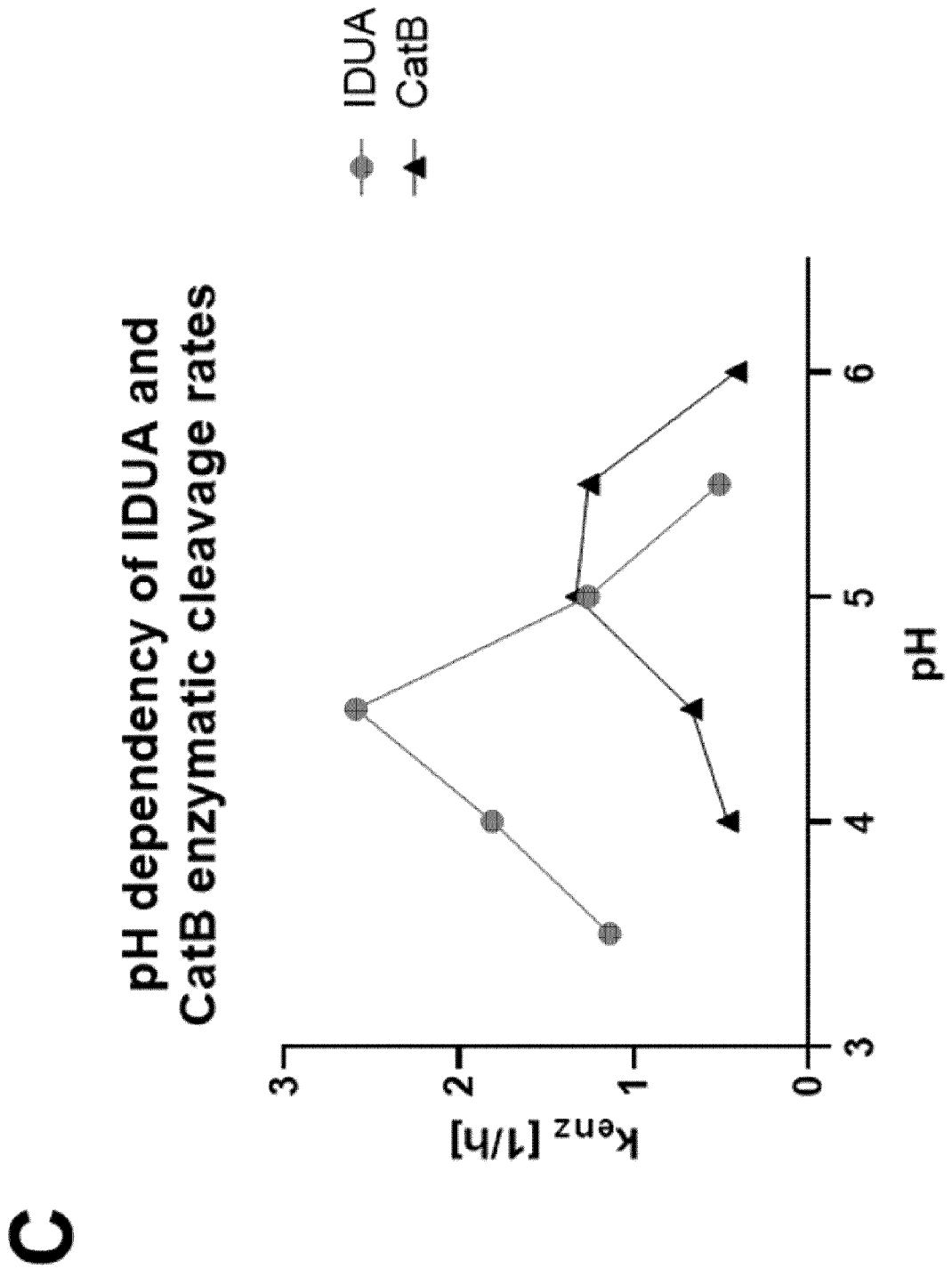

C
pH dependency of IDUA and
CatB enzymatic cleavage rates
IDUA
CatB
k_{enz} [1/h]
0
1
2
3
3
4
5
6
pH

Fig. 4 C

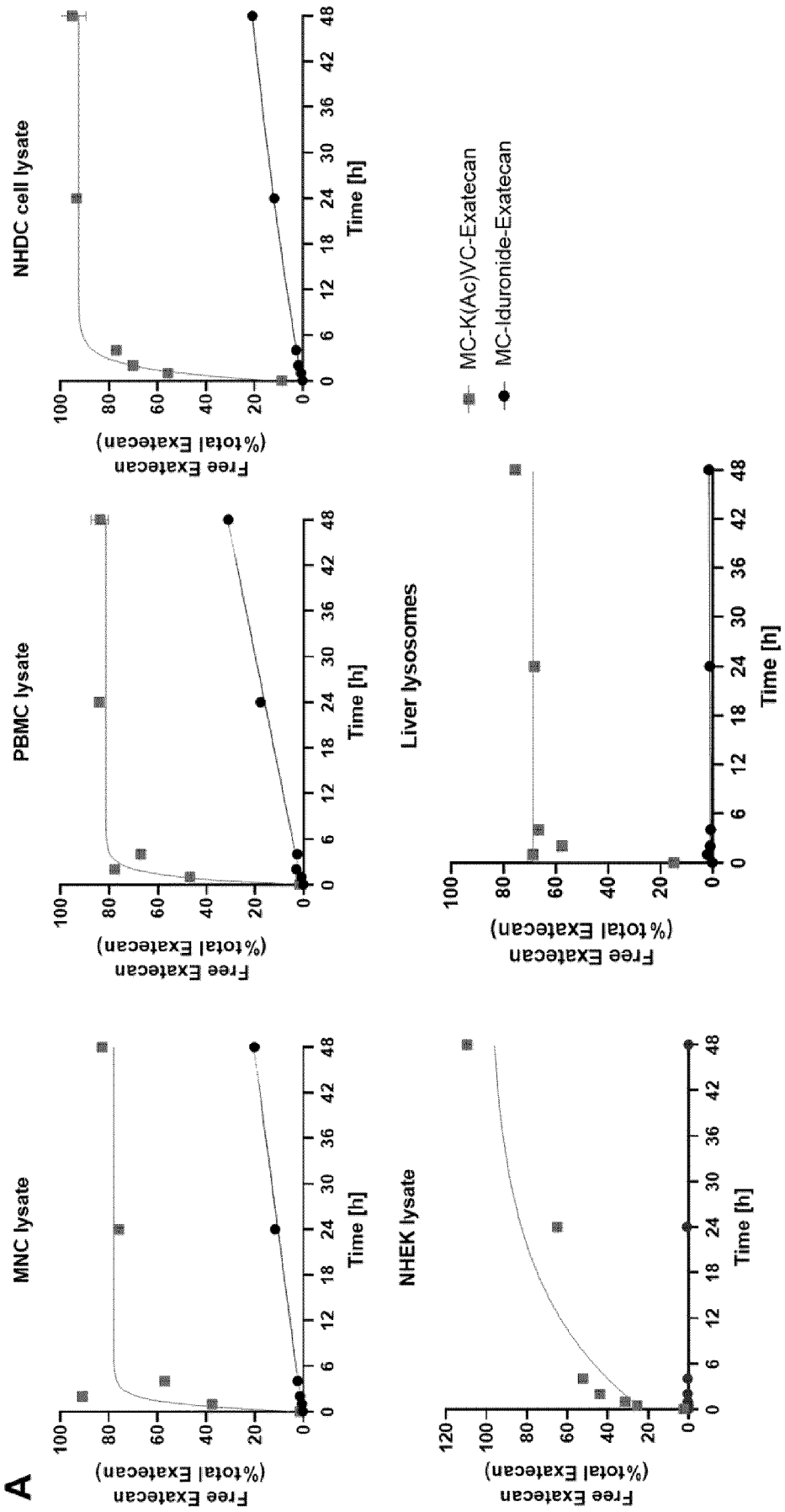

A
MNC lysate
NHEK lysate
PBMC lysate
Liver lysosomes
NHDC cell lysate
Free Exatecan (%total Exatecan)
Time [h]
MC-K(Ac)VC-Exatecan
MC-Iduronide-Exatecan

Fig. 5 A

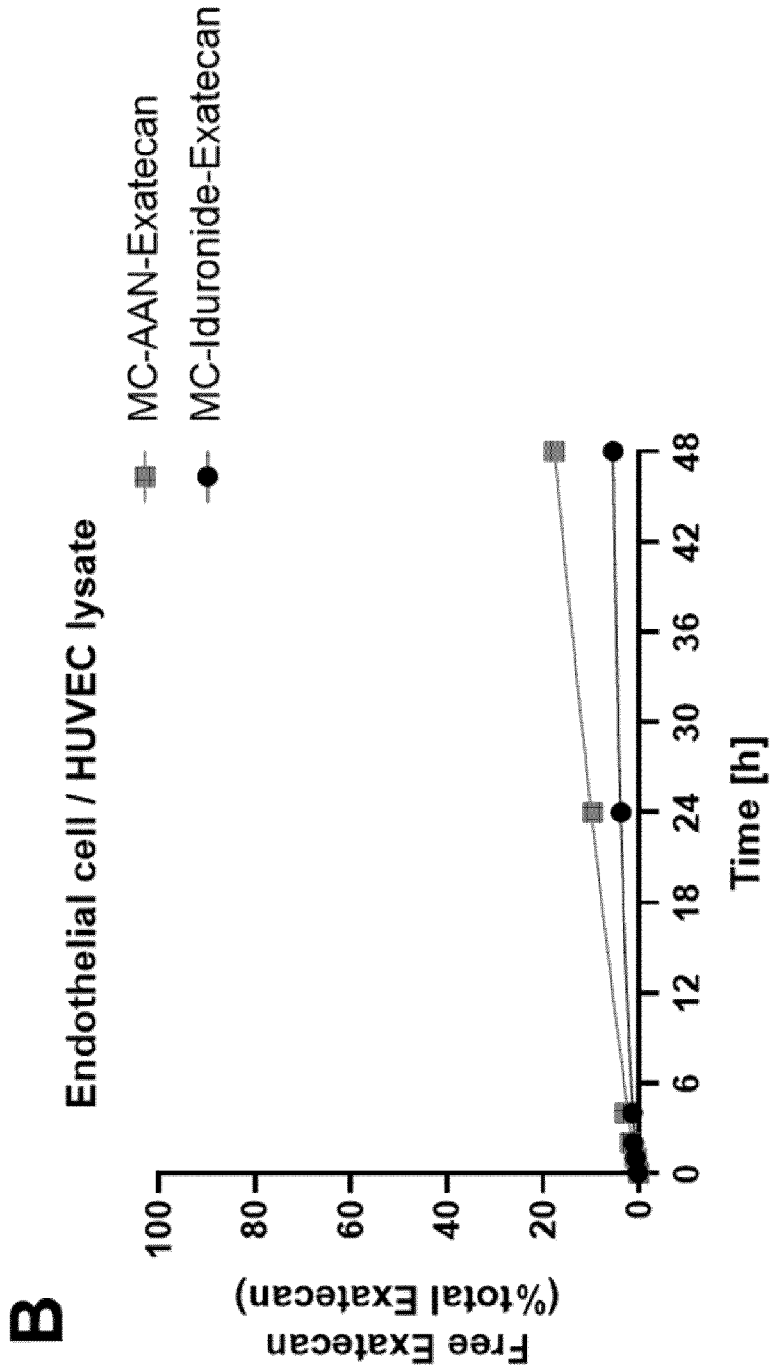
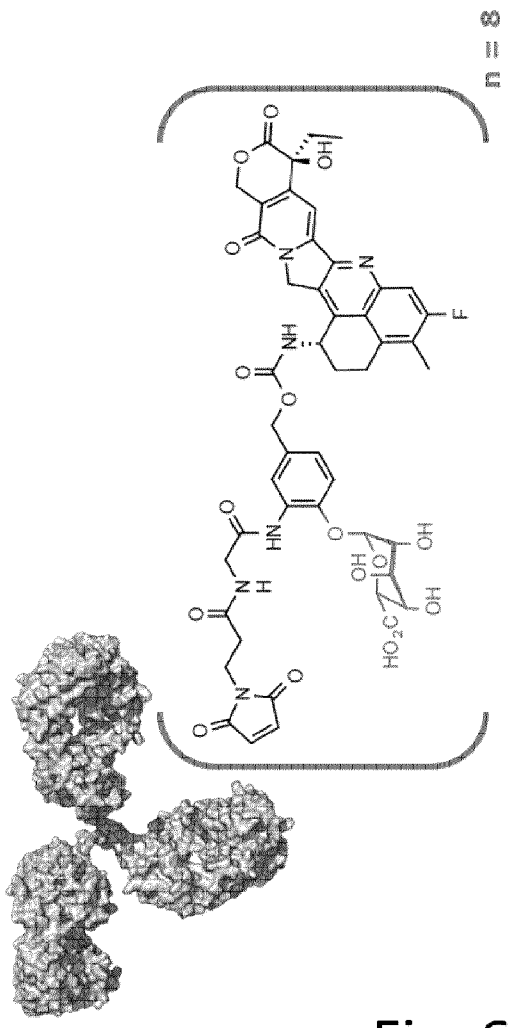
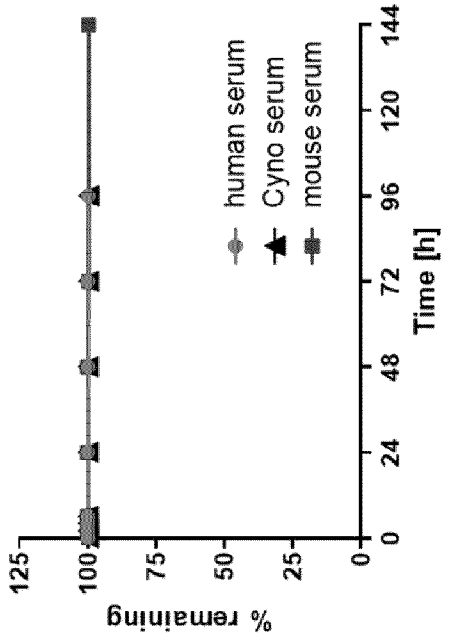

B
Endothelial cell / HUVEC lysate
MC-AAN-Exatecan
MC-Iduronide-Exatecan
Free Exatecan (%total Exatecan)
100
80
60
40
20
0
0
6
12
18
24
30
36
42
48
Time [h]

Fig. 5 B

A



C



B

name	mAb	Payload-Linker	Conjugation site	DAR	HIC RRT	SE-HPLC Purity [%]
aCEA5-Iduronide-Exatecan	aCEA5	Iduronide-Exatecan	Interchain Cys	7.8	1.05	99.8
aCEA5-K(Ac)VC-Exatecan	aCEA5	K(Ac)VC-Exatecan	Interchain Cys	8.0	1.38	93.6
aCEA5-AAN-Exatecan	aCEA5	AAN-Exatecan	Interchain Cys	7.7	1.22	96.9
aCEA5-Iduronide-Duocarmycin	aCEA5	Iduronide-Duocarmycin	Q295	2.0	1.09	98.4

Fig. 6

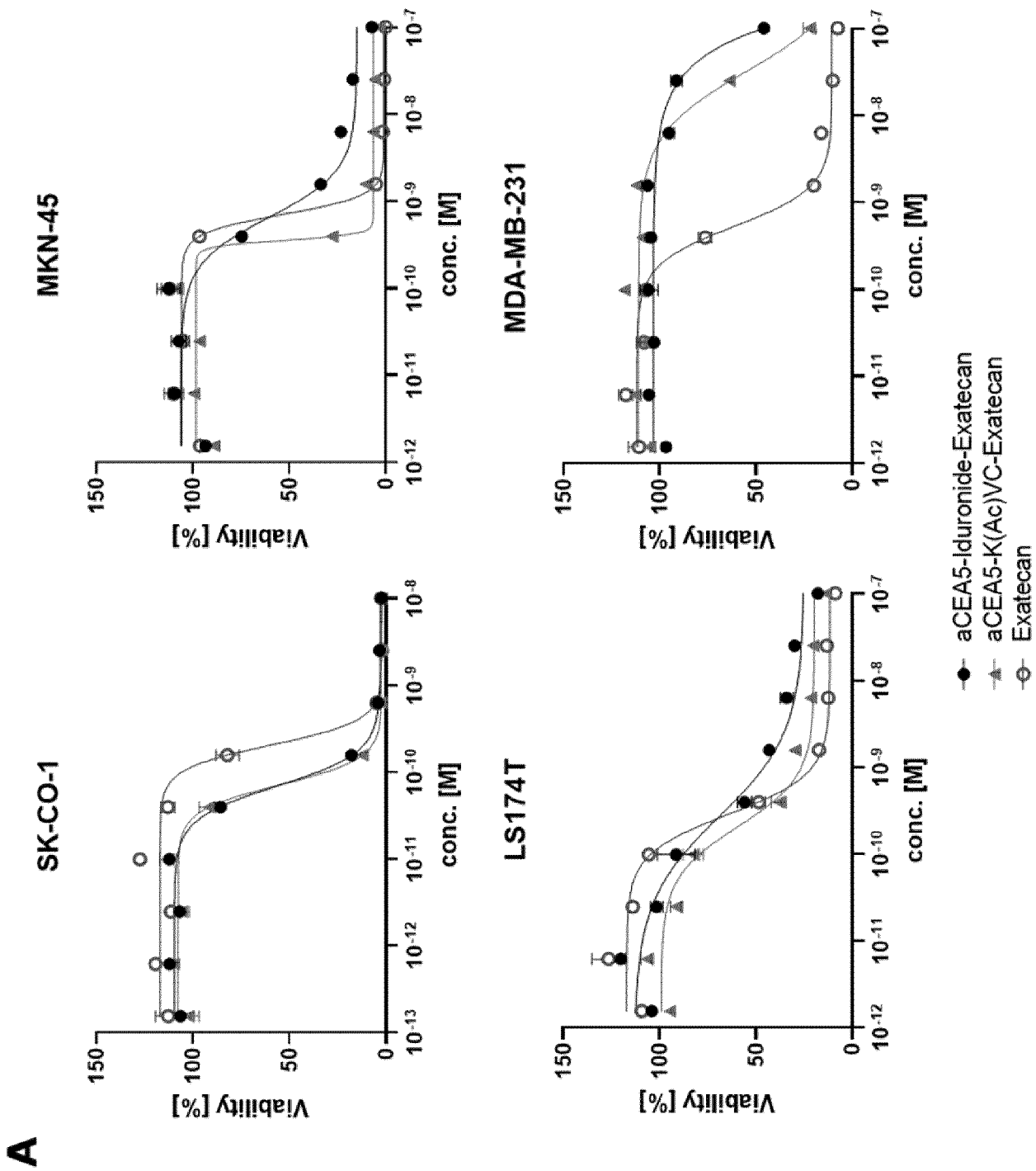

A
SK-CO-1
MKN-45
LS174T
MDA-MB-231
Viability [%]
conc. [M]
aCEA5-Iduronide-Exatecan
aCEA5-K(Ac)VC-Exatecan
Exatecan

Fig. 7 A

B

	IC_{50} [nM]			
	SK-CO-1	MKN-45	LS174T	MDA-MB-231
aCEA5-Iduronide-Exatecan	0.07 ± 0.01	0.65 ± 0.33	0.27 ± 0.25	~ 100
aCEA5-K(Ac)VC-Exatecan	0.07 ± 0.01	0.35 ±	0.20 ± 0.80	31.7 ± 9.2
Exatecan	0.20 ± 0.04	0.70 ± 0.24	0.28 ± 0.05	0.53 ± 0.09

Fig. 7 B

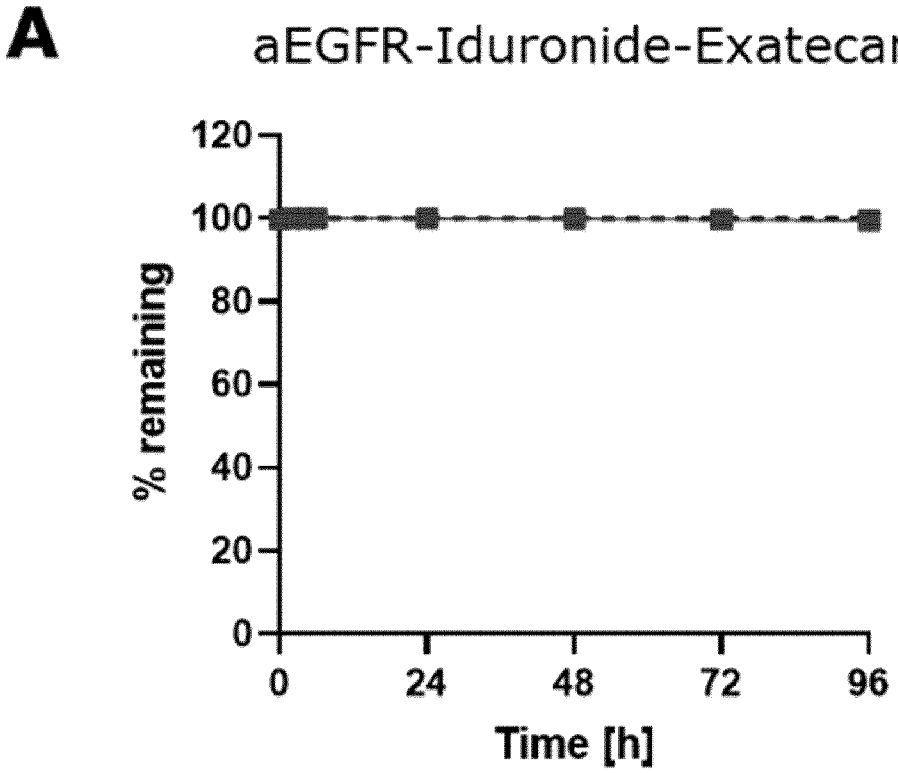
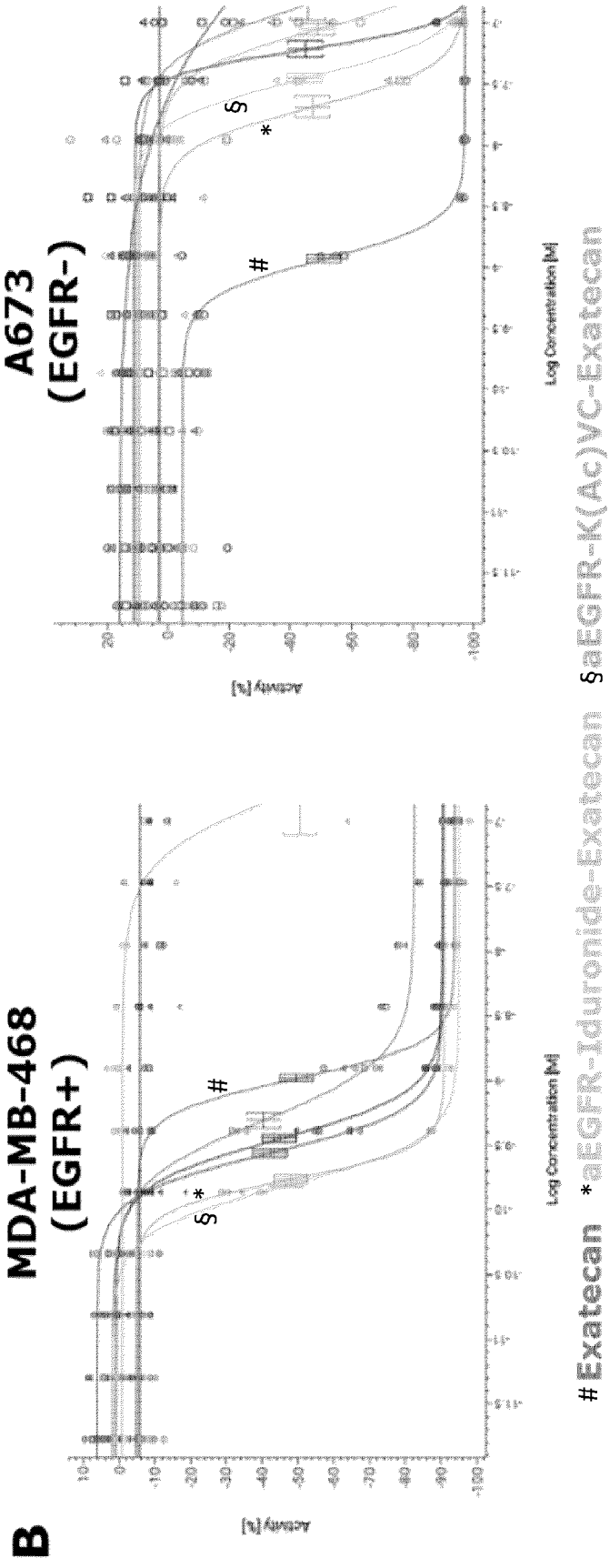

A
aEGFR-Iduronide-Exatecan
120
100
80
60
40
20
0
% remaining
0
24
48
72
96
Time [h]

Fig. 8 A



	IC_{50} [nM]	
	MDA-MB-468	A673
aEGFR-Iduronide-Exatecan	0.17	20.6
aEGFR-K(Ac)VC-Exatecan	0.15	35.1
Exatecan	1.0	1.2

Fig. 8 B

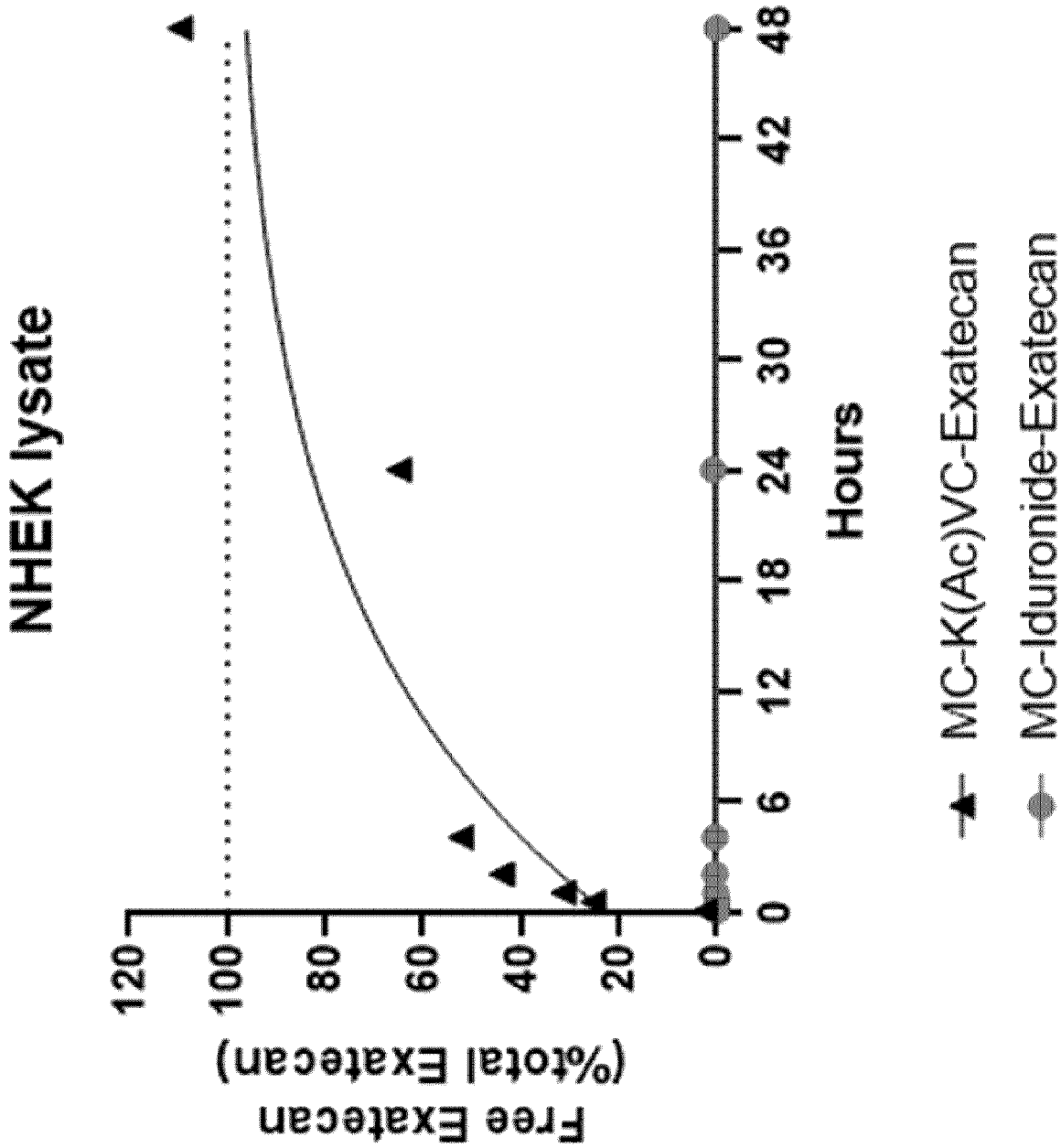
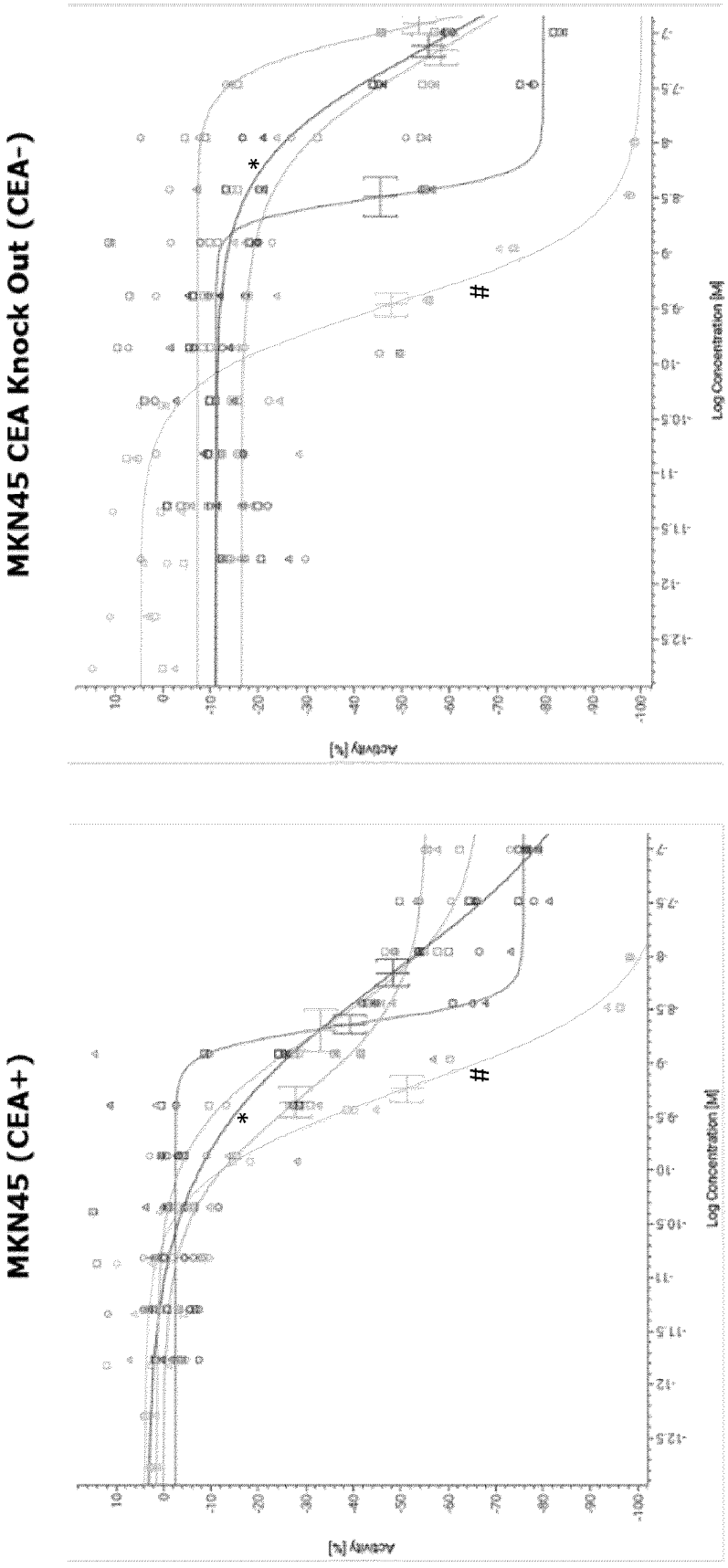

NHEK lysate
Free Exatecan (%total Exatecan)
120
100
80
60
40
20
0
0
6
12
18
24
30
36
42
48
Hours
MC-K(Ac)VC-Exatecan
MC-Iduronide-Exatecan

Fig. 9



	IC_{50} [nM]	
	MKN45	MKN45 CEA KO
aCEA5-Iduronide-Duocarmycin	2.07	70
Duocarmycin	0.59	0.34

Fig. 10

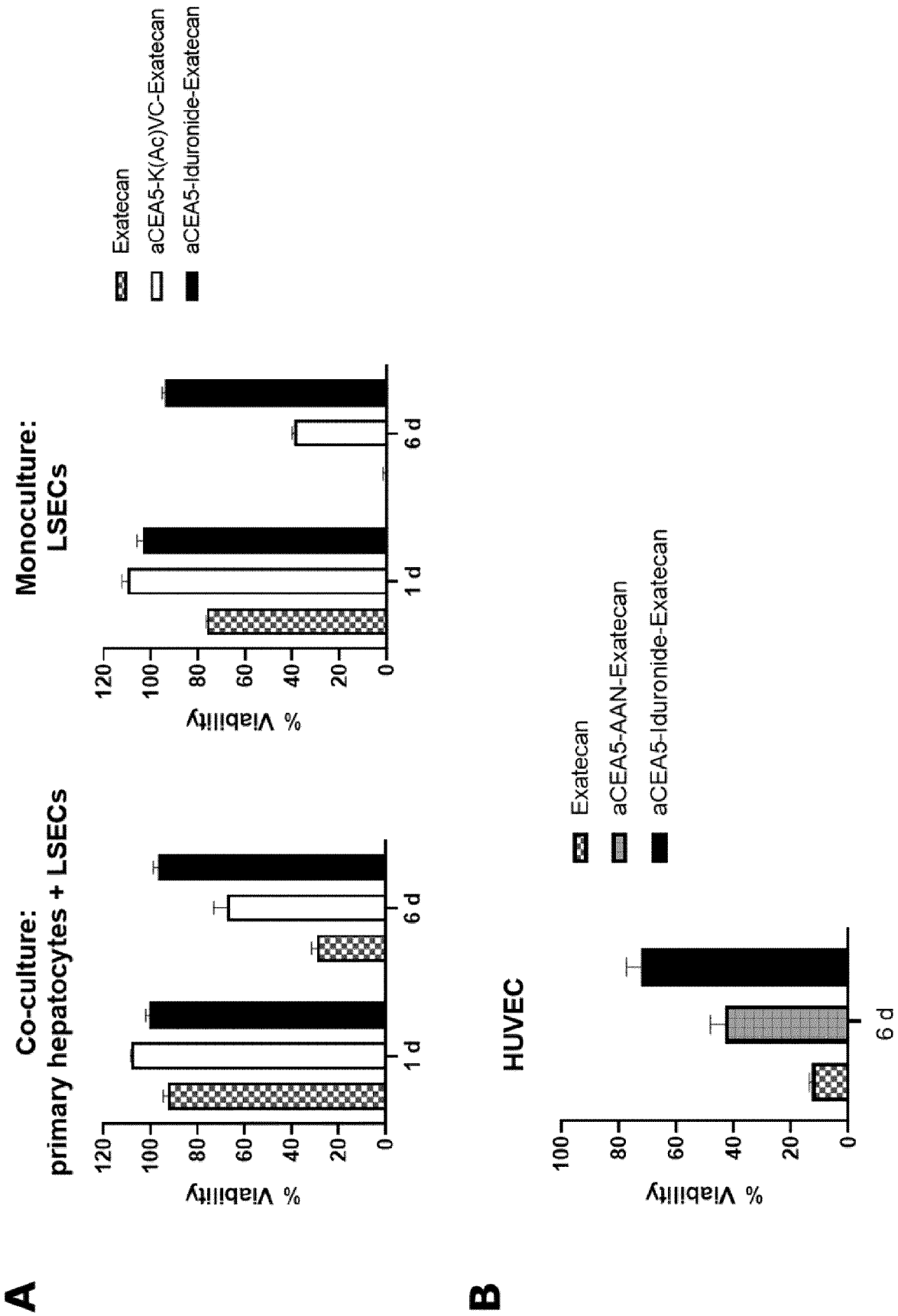

A
Co-culture:
primary hepatocytes + LSECs
Monoculture:
LSECs
Exatecan
aCEA5-K(Ac)VC-Exatecan
aCEA5-Iduronide-Exatecan
% Viability
0
20
40
60
80
100
120
1 d
6 d
B
HUVEC
Exatecan
aCEA5-AAN-Exatecan
aCEA5-Iduronide-Exatecan
% Viability
0
20
40
60
80
100
6 d

Fig. 11

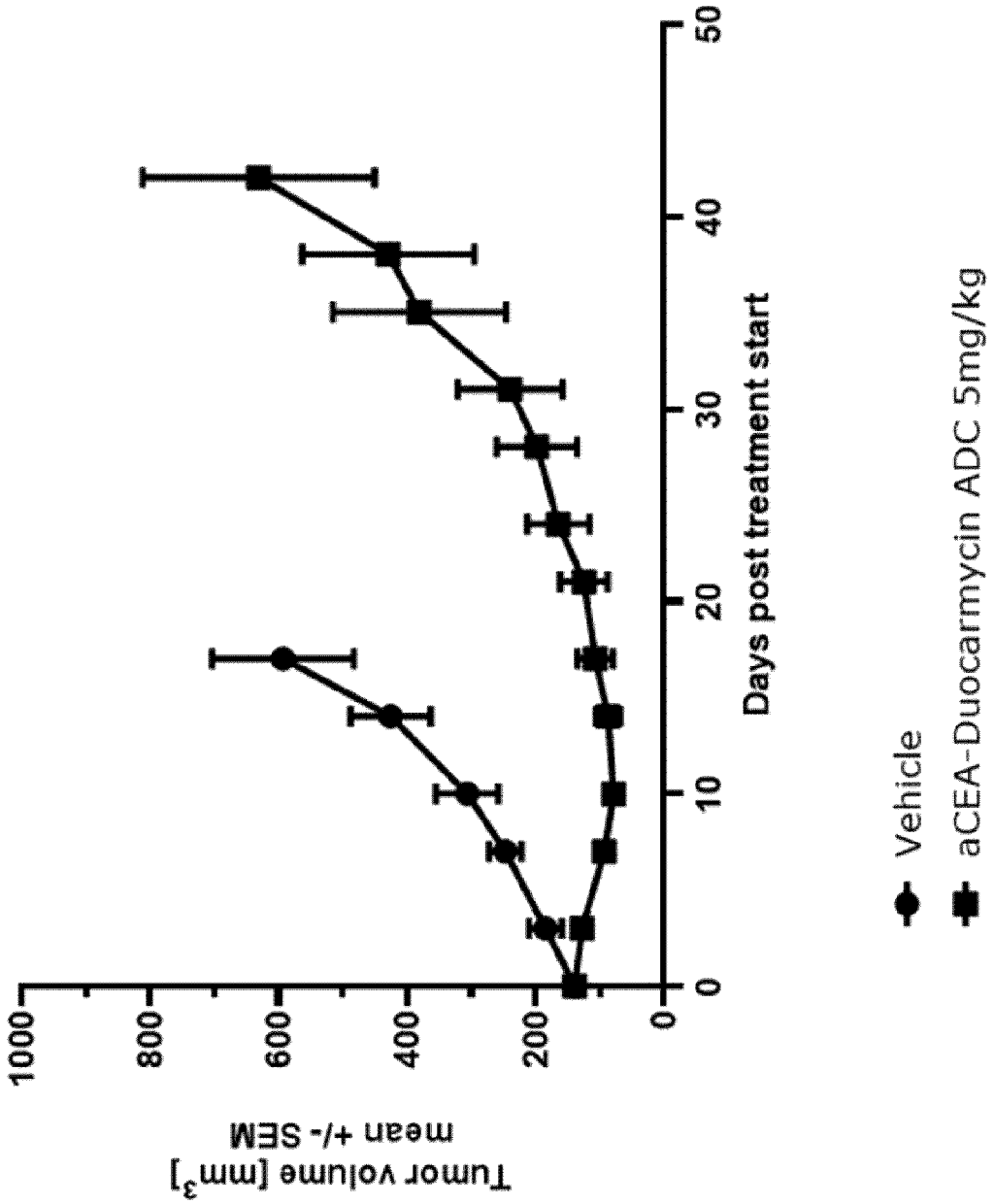

Tumor volume [mm³]
mean +/- SEM
1000
800
600
400
200
0
0
10
20
30
40
50
Days post treatment start
Vehicle
aCEA-Duocarmycin ADC 5mg/kg

Fig. 12

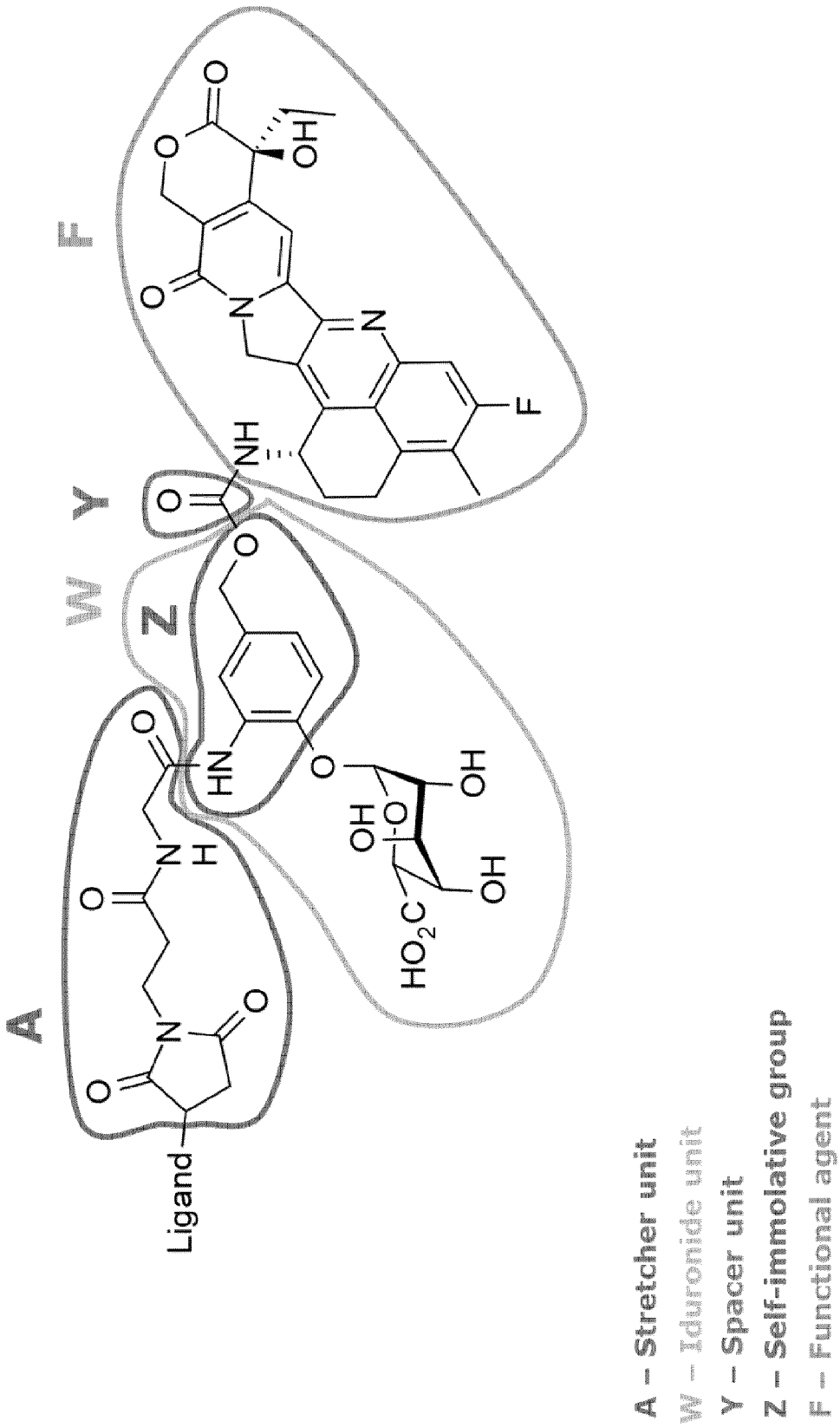

A
W
Y
Z
F
Ligand
A – Stretcher unit
W – Iduronide unit
Y – Spacer unit
Z – Self-immolative group
F – Functional agent

Fig. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/071143

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/68 A61K47/54 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCOTT C. JEFFREY ET AL: "Expanded Utility of the &bgr;-Glucuronide Linker: ADCs That Deliver Phenolic Cytotoxic Agents", ACS MEDICINAL CHEMISTRY LETTERS, vol. 1, no. 6, 9 September 2010 (2010-09-09), pages 277-280, XP055225196, US ISSN: 1948-5875, DOI: 10.1021/ml100039h CAS RN 1234476-13-1 page 278; figure 1; table 1 ----- -/--	1-7,9-15

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 September 2024	04/10/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Langer, Miren

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L	CHUANG HUAI-YAO ET AL: "Toward reducing immunogenicity of enzyme replacement therapy: altering the specificity of human [beta]-glucuronidase to compensate for [alpha]-iduronidase deficiency", PROTEIN ENGINEERING, DESIGN AND SELECTION, vol. 28, no. 11, 1 November 2015 (2015-11-01), pages 519-530, XP093207975, ISSN: 1741-0126, DOI: 10.1093/protein/gzv041 Retrieved from the Internet: URL:https://academic.oup.com/peds/article-pdf/28/11/519/17494969/gzv041.pdf> abstract -----	1-15
X	WO 2022/011075 A1 (VELOSBIO INC [US]; LANNUTTI BRIAN [US] ET AL.) 13 January 2022 (2022-01-13) CAS RN 2758292-53-2; claims 10, 11 -----	1-15
X	SERGII KOLODYCH ET AL: "Development and evaluation of &bgr;-galactosidase-sensitive antibody-drug conjugates", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, 1 August 2017 (2017-08-01), XP055416440, AMSTERDAM, NL ISSN: 0223-5234, DOI: 10.1016/j.ejmech.2017.08.008 figure 1 -----	1-7,9-15
X	WO 2018/090045 A1 (CHO PHARMA INC; LIN NAN HORNG [US]) 17 May 2018 (2018-05-17) CAS RN 2226610-90-6DP, 2226610-95-1DP, 2226611-12-5DP; claim 8 -----	1-15

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/071143

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	JÄGER SEBASTIAN ET AL: "Generation and Characterization of Iduronidase-Cleavable ADCs", BIOCONJUGATE CHEMISTRY, vol. 34, no. 12, 20 December 2023 (2023-12-20), pages 2221-2233, XP093207822, US ISSN: 1043-1802, DOI: 10.1021/acs.bioconjchem.3c00363 Retrieved from the Internet: URL:https://epo.summon.serialssolutions.com/2.0.0/link/0/eLvHCXMwpV3dS9xAEB_Uou2LrR_Vs1bXF5-MbrK7SQ6OwjV6V_EQEUV8CtmPQD8uJ8Yr9L_vTD7OVihSfAuTZHczs7szk9n5DYAIDrn3ZE9APS6yMORa-BoVsLBSCZXlnGcmNDWC_m0ir4bR5VCO5qDXpsbgmEpsuKxi-rTI72zeAA74R0TXXyfoN37DDxwfCkPoKmIeXqnIl-SJDc9Hjwi8flyHPgmTM6ZjPbvPNEQKy5R_K6x_WKG> the whole document	1-15

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/071143

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022011075 A1	13-01-2022	EP 4178559 A1	17-05-2023
		US 2023293712 A1	21-09-2023
		WO 2022011075 A1	13-01-2022
WO 2018090045 A1	17-05-2018	CN 109996543 A	09-07-2019
		EP 3538098 A1	18-09-2019
		TW 201818974 A	01-06-2018
		TW 202200213 A	01-01-2022
		US 2018133340 A1	17-05-2018
		US 2019328895 A1	31-10-2019
		WO 2018090045 A1	17-05-2018